PB80-181134

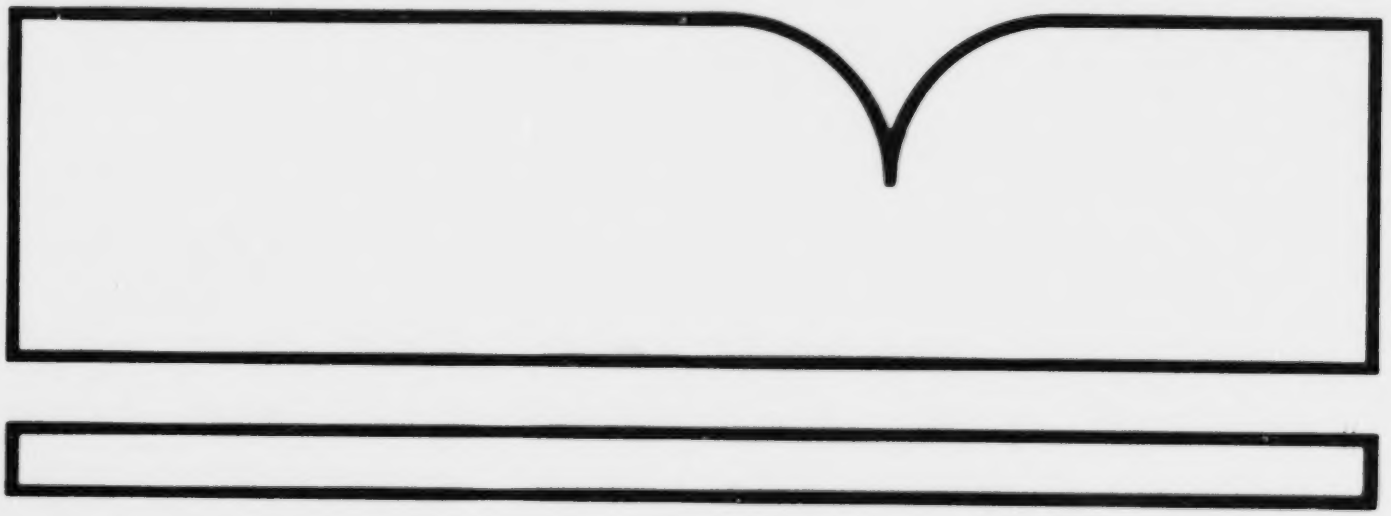
Treatability and Assessment of Coal Conversion Wastewaters. Phase I

North Carolina Univ. at Chapel Hill

Prepared for

Industrial Environmental Research Lab.
Research Triangle Park, NC

Nov 79



U.S. Department of Commerce
National Technical Information Service
NTIS

PB80-181134

United States
Environmental Protection
Agency

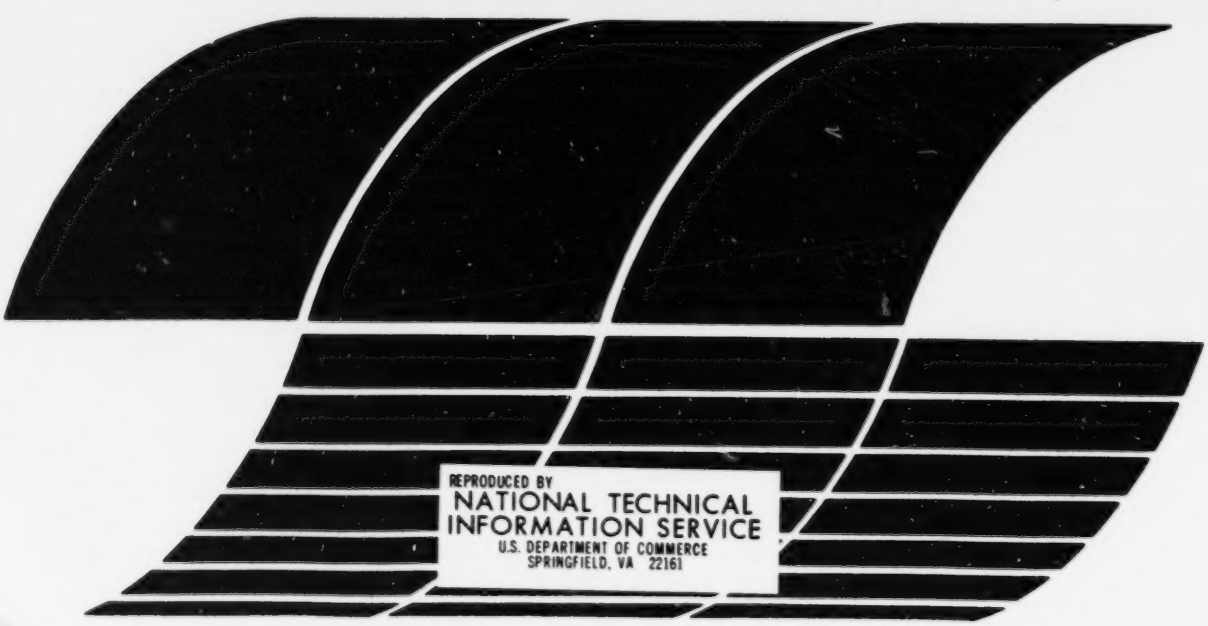
Industrial Environmental Research
Laboratory
Research Triangle Park NC 27711

EPA-600/7-79-248
November 1979

EPA

Treatability and Assessment of Coal Conversion Wastewaters: Phase I

Interagency Energy/Environment R&D Program Report



RESEARCH REPORTING SERIES

Research reports of the Office of Research and Development, U.S. Environmental Protection Agency, have been grouped into nine series. These nine broad categories were established to facilitate further development and application of environmental technology. Elimination of traditional grouping was consciously planned to foster technology transfer and a maximum interface in related fields. The nine series are:

1. Environmental Health Effects Research
2. Environmental Protection Technology
3. Ecological Research
4. Environmental Monitoring
5. Socioeconomic Environmental Studies
6. Scientific and Technical Assessment Reports (STAR)
7. Interagency Energy-Environment Research and Development
8. "Special" Reports
9. Miscellaneous Reports

This report has been assigned to the INTERAGENCY ENERGY-ENVIRONMENT RESEARCH AND DEVELOPMENT series. Reports in this series result from the effort funded under the 17-agency Federal Energy/Environment Research and Development Program. These studies relate to EPA's mission to protect the public health and welfare from adverse effects of pollutants associated with energy systems. The goal of the Program is to assure the rapid development of domestic energy supplies in an environmentally-compatible manner by providing the necessary environmental data and control technology. Investigations include analyses of the transport of energy-related pollutants and their health and ecological effects; assessments of, and development of, control technologies for energy systems; and integrated assessments of a wide range of energy-related environmental issues.

EPA REVIEW NOTICE

This report has been reviewed by the participating Federal Agencies, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Government, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

This document is available to the public through the National Technical Information Service, Springfield, Virginia 22161.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/7-79-248	2.
3. RECIPIENT'S ACCESSION NO. PB80 181134	
4. TITLE AND SUBTITLE Treatability and Assessment of Coal Conversion Wastewaters: Phase I	5. REPORT DATE November 1979
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) P. C. Singer, J. C. Lamb III, F. K. Pfaender, and R. Goodman	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS University of North Carolina--Chapel Hill Department of Environmental Sciences and Engineering Chapel Hill, North Carolina 27514	10. PROGRAM ELEMENT NO. EHE623A
	11. CONTRACT/GRANT NO. Grant No. R804917
12. SPONSORING AGENCY NAME AND ADDRESS EPA, Office of Research and Development Industrial Environmental Research Laboratory Research Triangle Park, NC 27711	13. TYPE OF REPORT AND PERIOD COVERED Final; 9/78 - 9/79
	14. SPONSORING AGENCY CODE EPA/600/13

15. SUPPLEMENTARY NOTES IERL-RTP project officer is N. Dean Smith, Mail Drop 61, 919/541-2708.

16. ABSTRACT The report gives Phase I results of (1) an assessment of the environmental impact of wastewaters originating from the production of synthetic fuels from coal, and (2) an evaluation of alternative technologies for treating these wastewaters. Work on coagulation, adsorption, and preliminary biological treatment studies is continuing. Future reports, representing successive phases, will update these results. The major focus is on aerobic biological treatment which is projected to be the principal means of removing organic impurities from these wastewaters and a cornerstone of any overall wastewater treatment program. A synthetic wastewater, designed to simulate a real conversion process wastewater, was fed to a series of aerobic biological reactors. Design and operation of the reactors is described, along with performance data spanning two 6-month operating periods. In addition to TOC, BOD, and COD data, the treated wastewaters were analyzed for phenolic content and residual organics, using chromatographic techniques. Aquatic bioassays and mammalian cytotoxicity tests were performed on the raw and treated wastewaters to evaluate their potential environmental impact.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS		b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group	
Pollution	Organic Compounds	Pollution Control	13B	07C
Coal	Bioassay	Stationary Sources	08G,21D	06A
Coal Gasification	Toxicity	Coal Conversion	13I	06T
Waste Water	Cytology	Synthetic Fuels		
Assessments			14B	
Water Treatment				
Aerobic Processes			06C	

18. DISTRIBUTION STATEMENT Release to Public	19. SECURITY CLASS *(This Report)* Unclassified	21. NO. OF PAGES
	20. SECURITY CLASS *(This page)* Unclassified	22. PRICE

EPA Form 2220-1 (9-73)

EPA-600/7-79-248

November 1979

Treatability and Assessment of Coal Conversion Wastewaters: Phase I

by

P.C. Singer, J.C. Lamb III,
F.K. Pfaender, and R. Goodman

University of North Carolina - Chapel Hill
Department of Environmental Sciences and Engineering
Chapel Hill, North Carolina 27514

Grant No. R804917
Program Element No. EHE623A

EPA Project Officer: N. Dean Smith

Industrial Environmental Research Laboratory
Office of Environmental Engineering and Technology
Research Triangle Park, NC 27711

Prepared for

U.S. ENVIRONMENTAL PROTECTION AGENCY
Office of Research and Development
Washington, DC 20460

ABSTRACT

The objectives of this project are to assess the environmental impact of wastewaters originating from the production of synthetic fuels from coal and to evaluate various technologies for the treatment of these wastewaters. The major focus to date has been on aerobic biological treatment which is projected to be the principal means of removing organic impurities from these wastewaters and a cornerstone of any overall wastewater treatment program.

A synthetic wastewater, designed to simulate a real conversion process wastewater, was formulated and fed to a series of aerobic biological reactors. Design and operation of the reactors is described, along with performance data spanning two six-month periods of operation. In addition to TOC, BOD, and COD data, the treated wastewaters were analyzed with respect to their phenolic content and the presence of residual organics using chromatographic techniques. Aquatic bioassays and mammalian cytotoxicity tests were performed on the raw and treated wastewaters to evaluate their potential environmental impact.

Experimental results from Phase I of the project, involving coagulation, adsorption, and preliminary biological treatability studies, are presented in this report. Model studies, using a simulated coal conversion wastewater at 25% of full strength, suggest that coal conversion wastewaters are biologically treatable via aerobic treatment processes;

phenol is completely removed by biological systems with sludge ages greater than 5 days. Preliminary aquatic bioassay experiments and cytotoxicity bioassays show that the toxicity of the wastewater is substantially reduced following biological treatment and that the reduction in toxicity increases with increasing sludge age. The adsorbability of the residual organics following biological treatment on activated carbon was found to decrease with increasing sludge age. Acidification and coagulation studies indicated that tar can be effectively precipitated and removed from coal conversion wastewaters by acidification to pH 4.5 to 5.0; coagulation using alum appears to be an ineffective means of removing tar from these wastewaters. Work on these tasks is continuing, and future reports representing successive phases of the project will update these results.

This report was submitted in partial fulfillment of the requirements of Grant No. R804917 by the Department of Environmental Sciences and Engineering of the University of North Carolina at Chapel Hill under the sponsorship of the U. S. Environmental Protection Agency. This report covers the period June 1, 1978 to September 30, 1979.

CONTENTS

		Page
Abstract		iii
Figures		vii
Tables		xii
Acknowledgements		xiv
1.	Introduction	1
2.	Conclusions	4
3.	Pretreatment of Coal Conversion Wastewaters for the Removal of Tars and Oils	6
	Experimental Methods	10
	Results and Discussion	17
	Conclusions	36
4.	Biological Treatment of Synthetic Coal Conversion Wastewaters: Part 1	37
	Formulation of Synthetic Coal Conversion Wastewater	38
	Description of Pilot Units	39
	Operation of Pilot Units	43
	Preliminary Results	45
	Summary of Preliminary Results	53
5.	Biological Treatment of Synthetic Coal Conversion Wastewaters: Part 2	56
	Operating Procedures	56
	Results	60
6.	Kinetic Analysis of Biological Treatability Data	82

7. Specific Organic Analysis and Environmental Assessment of Treated Wastewaters: Preliminary Results..... 98

HPLC Analysis..... 98
Aquatic Bioassay..... 110
Health Effects Bioassay..... 122

8. Volatility and Air-Stripping of Organics During Biological Treatment..... 127

Procedure..... 127
Results and Discussion..... 128
Conclusions..... 129

9. Biodegradability of Coal Conversion Wastewater Constituents..... 133

Procedure..... 134
Calculations and Results..... 135
Discussion..... 143

10. Mixed Liquor Respiration Studies..... 145

11. Adsorption of Alkyl Phenols and Residual TOC Following Biological Treatment..... 160

Procedures..... 161
Results..... 164

References..... 175

FIGURES

Number		Page
1	Tar, Oil, and Grease Concentrations from Synthane Gasification of Illinois No. 6 Coal After Various Stages of Treatment....	9
2	Effect of Acidification on the Formation of Settleable Solids in Coal Gasification Wastewater.....	19
3	Effect of Acidification on the Removal of Acetone-Soluble Tar from Coal Gasification Wastewater.....	20
4	Effect of Acidification on TOC Removal from Coal Gasification Wastewater.....	23
5	Effect of Acidification on COD Removal from Coal Gasification Wastewater.....	24
6	Titration of Coal Gasification Wastewater with Sulfuric Acid.....	25
7	Removal of Acetone-Soluble Tar by Coagulation with Alum at pH 5.7.....	27
8	Removal of TOC by Coagulation with Alum.....	28
9	TOC Removal by Acidification in the Presence and Absence of Alum.....	29
10	Production of Settleable Solids Resulting from Alum Coagulation at pH 5.7.....	31
11	Effect of Polymer (DEAE-Dextran) Addition on the Removal of Acetone-Soluble Tar at pH 6.0.....	32
12	Effect of Polymer (DEAE-Dextran) Addition on the Removal of TOC at pH 6.0.....	33
13	Diagram of Experimental Biological Reactors.....	41
14	Performance Characteristics of the 5-Day Reactor.....	46
15	Performance Characteristics of the 10-Day Reactor.....	47

16	Performance Characteristics of the First 20-Day Reactor.....	48
17	Performance Characteristics of the Second 20-Day Reactor....	49
18	Effect of Residence Time on Reactor Performance and Stability.....	54
19	Effluent TOC and Mixed Liquor Volatile Suspended Solids Concentration in 5-Day Reactor.....	62
20	Effluent TOC and Mixed Liquor Volatile Suspended Solids Concentration in 7.5-Day Reactor.....	63
21	Effluent TOC and Mixed Liquor Volatile Suspended Solids Concentration in 10-Day Reactor.....	64
22	Effluent TOC and Mixed Liquor Volatile Suspended Solids Concentration in First 20-Day Reactor.....	65
23	Effluent TOC and Mixed Liquor Volatile Suspended Solids Concentration in Second 20-Day Reactor.....	66
24	Effluent TOC and Mixed Liquor Volatile Suspended Solids Concentration in 40-Day Reactor.....	67
25	Correlation Between COD and TOC in Biologically-Treated Wastewater.....	71
26	Correlation Between BOD and TOC in Biologically-Treated Wastewater.....	72
27	Correlation Between BOD and COD in Biologically-Treated Wastewater.....	73
28	Effect of Residence Time on TOC Removal.....	77
29	Effect of Residence Time on COD Removal.....	78
30	Effect of Residence Time on BOD Removal.....	79
31	Effect of Residence Time on Phenols Removal.....	80
32	Relationship Between Solids Residence Time and TOC Loading..	86
33	Relationship Between Solids Residence Time and BOD Loading..	87
34	Relationship Between Solids Residence Time and COD Loading..	88
35	Kinetics of Substrate Utilization: BOD Basis.....	89
36	Kinetics of Substrate Utilization: TOC Basis.....	90

37	Kinetics of Substrate Utilization: COD Basis.....	91
38	Linearized Substrate Utilization Plot: BOD Basis.....	92
39	Linearized Substrate Utilization Plot: TOC Basis.....	93
40	Linearized Substrate Utilization Plot: COD Basis.....	94
41	HPLC Chromatographic Profiles of Raw Synthetic Wastewater...	100
42	HPLC Chromatographic Profiles of 5-Day Reactor Effluent.....	102
43	HPLC Chromatographic Profiles of 7.5-Day Reactor Effluent...	103
44	HPLC Chromatographic Profiles of 10-Day Reactor Effluent....	104
45	HPLC Chromatographic Profiles of 20-Day Reactor Effluent....	105
46	HPLC Chromatographic Profiles of 40-Day Reactor Effluent....	106
47	Identification and Quantitation of HPLC Chromatographic Peaks from 10-Day Reactor.....	108
48	Growth of Selenastrum capricornutum Exposed to Various Dilutions of Raw Synthetic Wastewater.....	116
49	Growth of Selenastrum capricornutum Exposed to Various Dilutions of 5-Day Reactor Effluent.....	117
50	Growth of Selenastrum capricornutum Exposed to Various Dilutions of 10-Day Reactor Effluent.....	118
51	Growth of Selenastrum capricornutum Exposed to Various Dilutions of 20-Day Reactor Effluent.....	119
52	Toxicity of Raw and Biologically-Treated Synthetic Wastewater to Fathead Minnows (Pimephales promelas).....	120
53	Toxicity of Raw and Biologically-Treated Synthetic Wastewater to Daphnia pulex.....	121
54	Results of 20-Hour Clonal Toxicity Assay Using V-79 Chinese Hampster Cells.....	124
55	HPLC Chromatographic Profile of Synthetic Wastewater for Stripping Evaluation.....	131
56	Rate of Oxygen Utilization Resulting From Microbial Degradation of Phenol.....	137

57	Rate of Oxygen Utilization Resulting from Microbial Degradation of p-Cresol	137
58	Rate of Oxygen Utilization Resulting from Microbial Degradation of o-Cresol	138
59	Rate of Oxygen Utilization Resulting from Microbial Degradation of m-Cresol	138
60	Rate of Oxygen Utilization Resulting from Microbial Degradation of 2,5-Dimethylphenol	139
61	Rate of Oxygen Utilization Resulting from Microbial Degradation of 2,3-Dimethylphenol	139
62	Rate of Oxygen Utilization Resulting from Microbial Degradation of 2,6-Dimethylphenol	140
63	Rate of Oxygen Utilization Resulting from Microbial Degradation of 3,5-Dimethylphenol	140
64	Rate of Oxygen Utilization Resulting from Microbial Degradation of 3,4-Diemthylphenol	141
65	Experimental Set-Up for Mixed Liquor Respiration Studies	146
66	Common Types of Oxygen Utilization Curves	148
67	Respiration Curves Using Original Oxygen Utilization Procedure	149
68	Respiration Rate of Aerated Sludge with 200 mg/l Phenol	151
69	Effect of Time on Respiration Rate with 2000 mg/l Phenol	154
70	Respiration Rate of "Fresh" Sludge from Reactor	155
71	Respiration Curves for Phenol Using Modified Oxygen Utilization Procedure on Selected Substrates	157
72	Results Using Modified Oxygen Utilization Procedure on Selected Substrates	158
73	Adsorption of Methylphenols by Activated Carbon	165
74	Adsorption of Dimethylphenols by Activated Carbon	166
75	Adsorption of Ethylphenols by Activated Carbon	167
76	Comparative Adsorption of Alkyl-Substituted Phenols by Activated Carbon	168

77 Adsorption of Phenol by Activated Carbon..... 170

78 Adsorption of Isopropylphenol by Activated Carbon..... 171

79 Adsorption of Raw and Biologically-Treated Synthetic Wastewater by Activated Carbon..... 173

TABLES

Number		Page
1	Typical Oil, Grease, and Tar Concentrations in Synthane By-Product Water	7
2	Typical Jar Test Results with an Illinois #6 Coal Wastewater Produced During Synthane Gasification	8
3	Physical and Chemical Characteristics of Coal Gasification Wastewater Sample	18
4	Results of Analysis of Residual Supernatant Layer After Acidification of Wastewater Sample to pH 4.8	26
5	Phenol Removal by Acidification and Coagulation	35
6	Composition of Synthetic Coal Conversion Wastewater	40
7	Summary of Reactor Performance During Periods of Intensive Analysis	51
8	Average Quality of Effluent from Biological Treatment Units.	55
9	Concentration of Inorganic Constituents in Quarter-Strength Wastewater	57
10	Record of Equipment Malfunctions	61
11	Summary of Reactor Performance	69
12	Periods of Steady State Performance	75
13	Summary of Average Steady State Reactor Performance	76
14	Calculated Process Loading Factors for Biological Reactors	84
15	Summary of Kinetic Coefficients	95
16	Kinetic Coefficients from Biological Treatment of Synthane and Synthoil Wastewater	97
17	Identification of HPLC Peaks	101

18	Concentrations of Major Phenolic Compounds in Reactor Effluents	109
19	Effects of Raw and Biologically-Treated Wastewaters on Fathead Minnows and Daphnia Pulex	113
20	Summary of Mammalian Cytotoxicity Data	125
21	Change in Total Organic Carbon Concentration Resulting from Aeration	129
22	Results of HPLC Analysis of Aerated Wastewater	130
23	Biodegradation of Selected Coal Gasification Wastewater Components	142
24	Effect of Aeration on "Activity" of Sludge	152
25	Characteristic Wavelengths for Maximum UV Absorbance of Aqueous Phenolic Solutions	162
26	Langmuirian Coefficients for the Adsorption of Alkyl Phenols on PX-21 Powdered Activated Carbon	172

ACKNOWLEDGEMENTS

We would like to acknowledge the help provided by Michael Hughes, Gerald Speitel, Dave Reckhow and Roger Rader in designing, constructing and operating the biological reactors; Randall Williams for carrying out the coagulation and acidification experiments; Chen Yen for performing the adsorption studies; Teena Cochran, Liz Anderson, Marynoel Monson and Anh Chan for their assistance in the analytical phases of the project; Anthony Maciorowski, Jane Hughes, Cecily Beall, and Steve Shoaf for their aquatic bioassay activities; Mark Sobsey, Randy Jones and Leslie McGeorge for setting up and performing the mammalian cytotoxicity assays; and Jolene Chinchilli and Dave Ruehle for carrying out the biodegradation studies.

The assistance provided by Drs. Dean Smith and Thomas Petrie, our Project Officers, and their colleagues at the Industrial Environmental Research Laboratory of the U. S. Environmental Protection Agency at Research Triangle Park, N.C., in guiding and facilitating the performance of this research is also appreciated.

SECTION 1

INTRODUCTION

In a previous review (1) of the characteristics of wastewaters generated as a result of the production of synthetic fuels from coal, it was shown that these wastewaters contain substantial amounts of organic substances, many of which can have an adverse impact on aquatic life and on human health. CODs (chemical oxygen demands) on the order of 10,000-40,000 mg/l and TOC (total organic carbon) concentrations of 5,000-10,000 mg/l have been reported (2, 4) for these wastewaters. Specific organic analysis of these wastes shows that 60-80% of the total organic carbon is phenolic in nature, consisting of monohydric, dihydric, and polyphenols. The remainder of the organic material identified consists of monocyclic and polycyclic nitrogen-containing aromatics, oxygen- and sulfur-containing heterocyclics, polynuclear aromatic hydrocarbons, and simple aliphatic acids. The composition of the wastewaters from various coal gasification and coal liquefaction processes appears to be relatively uniform, especially with respect to the phenolic constituents, regardless of the specific process technology or feed coal employed (1).

Aerobic biological processes appear to be the focal point of any overall scheme for treating these wastewaters since a significant number of the major constituents of the wastes are biodegradable. Accordingly, suitable design and operating criteria for biological treatment of these

wastewaters need to be developed. In order to establish such guidelines and to evaluate the efficacy of biological treatment, treatability studies need to be conducted.

Several research groups (3,4,5, and 6) have conducted and are currently performing biological treatability studies on wastewaters produced from a variety of pilot coal gasification and liquefaction facilities such as the Hygas, Synthane, Lurgi, and H-Coal processes. Accordingly, these treatability studies are closely bound to the conversion process being investigated. However, since coal conversion processes are still in the developmental stage, there is some question as to whether or not a suitable, consistent and representative wastewater could be obtained for a comprehensive analysis and assessment of overall coal conversion wastewater treatability. Hence, the studies described in this report have been conducted using a synthetic wastewater which has been formulated to be representative in its organic composition, of actual coal conversion wastewaters. The wastewater contains twenty-eight organic compounds, inorganic nutrients, and pH-buffers.

The synthetic coal conversion wastewater is being used to feed several bench-scale activated sludge reactors. In addition to generating acclimatized organisms for separate biodegradability studies of model compounds identified in actual coal conversion wastewaters, the pilot reactors are being used to treat the synthetic wastewater under various types of operating conditions. Effluents from the reactors are being analyzed by gas chromatography, gas chromatography/mass spectrometry, and high performance liquid chromatography to assess the degree of removal of the various constituents in the raw feed, and to identify reaction products

following biological treatment. Additionally, acute toxicity studies using fish, Daphnia, and algae are being conducted to evaluate the biological impact of the treated wastewaters on aquatic life. Acute mammalian cytotoxicity and Ames mutagenicity analyses are also being performed on the reactor effluents to assess their potential impact on human health. This report presents some of the initial results of the biological treatability evaluation, along with some additional studies directed at wastewater pretreatment for acidification and coagulation, and adsorption of phenolic substances and residual TOC from biologically-treated wastewater using activated carbon.

SECTION 2

CONCLUSIONS

Based upon preliminary model studies using a synthetic coal conversion wastewater at 25% of full strength and aerobic biological processes without solids recycle, coal conversion wastewaters appear to be biologically treatable. TOC, COD, and BOD removal increase with increasing sludge age (solids residence time) and reductions of 85 to 97%, 86 to 96%, and 99.8%, respectively, have been obtained with sludge ages of 20 days. Phenol is essentially completely removed with a sludge age of 5 days, while the cresols and xylenols require 7.5 to 10 days and 20 days, respectively, for removal to levels below 1 mg/l. Although some difficulties were encountered in achieving stable reactor operation and steady state performance, the TOC, COD, and BOD data appear to be in conformance with commonly accepted kinetic models; the kinetic coefficients for microbial growth and substrate utilization using the synthetic wastewater are of the same order and essentially in agreement with the results of other investigators performing biological treatability studies on samples of real wastewaters generated from developing coal conversion technologies.

Preliminary aquatic bioassay experiments with fish, Daphnia and algae, and a health effects acute cytotoxicity bioassay employing mammalian cells exposed to raw and biologically treated synthetic coal conversion wastewaters show that toxicity of the waste is substantially reduced

following biological treatment, and that the reduction in toxicity increases with increasing sludge age. These bioassays are continuing in an attempt to develop more definitive data with respect to the biological impact of the treated wastewaters.

Activated carbon adsorption studies show that alkyl-substituted phenols are more strongly adsorbed than phenol and that the extent of adsorption increases as the number of alkyl substituents and the length of the alkyl chain increase. Position of the substituent alkyl group has no effect on the extent of adsorption. With respect to the adsorbability of the residual organic carbon following biological treatment, the extent of adsorption appears to decrease with increasing sludge age of the biological system. Interpretation of the results is based upon the polar nature (aqueous solubility) of the residual organic compounds comprising the effluent TOC from the reactors.

Acidification and coagulation studies show that tar can be effectively precipitated and removed from real coal conversion wastewaters by acidification of the waste to pH 4.5 to 5. Coagulation using alum appeared to be an ineffective means of removing tar from these wastewaters.

These treatability studies are continuing, as are chemical and biological assessment studies of the effluents following various degrees of treatment. Particular attention will be directed at priority pollutants present in these wastewaters. The results of these additional studies will be available in the next report in this series. Ultimately, the conclusions developed using the synthetic coal conversion wastewater will be tested on real conversion wastewaters.

SECTION 3

PRETREATMENT OF COAL CONVERSION WASTEWATERS FOR THE REMOVAL OF TARS AND OILS

Wastewaters produced from coal gasification pilot plants have been found to contain suspended material such as tar and oil as well as dissolved organic compounds (2). These tars and oils are composed of an organic matrix of compounds including some carcinogenic polycyclic aromatic hydrocarbons and their derivatives. The presence of tar and oil in coal gasification wastewaters can contribute to the fouling of equipment and pipes and, in addition, has been shown to interfere with aerobic biological tre tment processes for coke plant wastewaters (7).

Johnson et al. (4) have published the results of experiments designed to describe the removal of tar and oil from Synthane coal gasification wastewaters using different coagulants. Table 1 gives typical concentrations of oil and grease, and acetone-soluble tar found in Synthane by-product water. These analyses apply to the decanted supernatant layer from the water-cooled condensers. The raw condensate, containing about 22,000 mg/l of tar, oil, and grease, was allowed to settle 3 to 6 hours before the supernatant was drawn off. The authors report that this layer is typical of the wastewater that would be sent to a treatment plant from a commercial Synthane facility.

TABLE 1. TYPICAL OIL, GREASE, AND TAR CONCENTRATIONS IN SYNTHANE BY-PRODUCT WATER* (All values in mg/l)

Type of Coal Gasified	Oil and Grease+	Acetone-Soluble Tar#
Montana Rosebud	910	1,180
Montana Rosebud	840	1,150
Illinois #6	1,020	1,970
Illinois #6	460	550

*After Johnson et al. (4)

+Freon-soluble materials measured according to Standard Methods (8).

#Does not include components measured as oil and grease.

Table 2 gives the results of jar tests performed with Synthane supernatant. The coal used in this particular run was Illinois #6 coal. Conditions (pH, alum dose, and polymer dose) of the experiments are given, yet no quantitative data are presented for evaluation of the different conditions applied. Figure 1 shows a tar reduction from 1,000 mg/l in the supernatant layer to 600 mg/l after treatment consisting of pH depression and alum addition followed by pH adjustment. The exact conditions of coagulant dose and pH are not specified. The removal of tar and oil from the supernatant was 47 percent for a total of 97 percent reduction from the raw condensate.

It appears that acidification and alum addition are effective means of removing tar and oil, but the appropriate conditions for achieving best removal are not clear. Quantitative data describing the removal of tar and

TABLE 2. TYPICAL JAR TEST RESULTS WITH AN ILLINOIS #6 COAL WASTEWATER PRODUCED DURING SYNTHANE GASIFICATION*

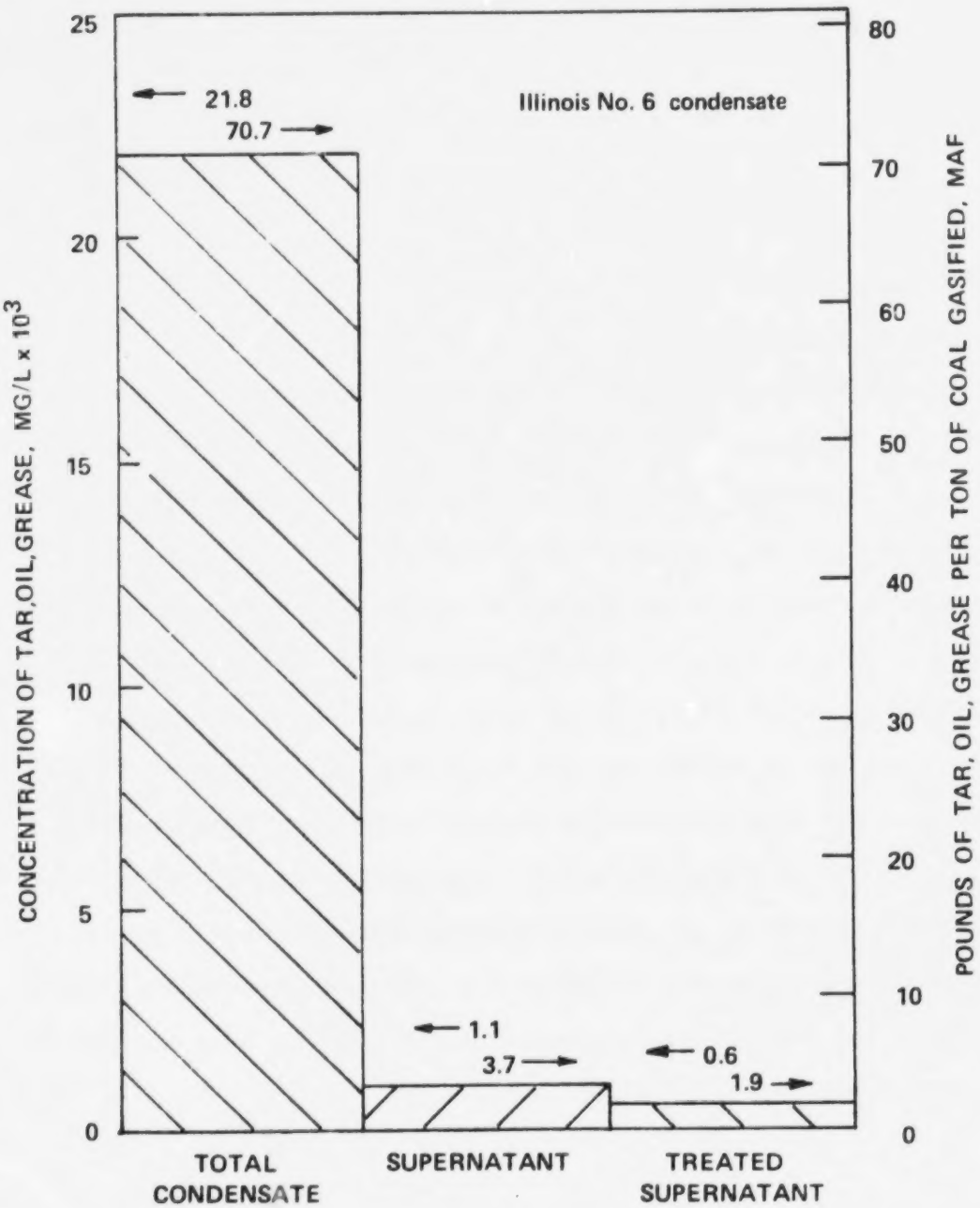
Jar #	Adjusted pH	pH After Neutralization	Alum (mg/l)	Observations
1+	1.9	7.0	25	Some surface oil and tar
2+	2.0	7.0	50	Some surface oil and tar
3+	3.5	7.0	25	Surface oil with some noticeable floc formation
4+	2.4	7.0	50	Considerable oil on surface, noticeable floc formation
5+	2.5	7.0	100	Much surface oil, excellent floc formation
6+	1.5	7.0	150	Much surface oil, opaque liquid, excellent floc formation
7#	6.9	---	20	Much surface oil, no floc
8#	6.8	---	40	Much surface oil, no floc
9#	6.7	---	60	Much surface oil, floc visible
10#	7.1	---	80	Much surface oil, floc visible
11#	6.9	---	100	Much surface oil, significant floc
12#	6.9	---	120	Much surface oil, less floc than in jar #11

*After Johnson et al. (4)

+Alum alone

#Alum and polymer; polymer dosage = 2 mg/l

Figure 1. Tar, oil, and grease concentrations from Synthane gasification of Illinois No. 6 coal after various stages of treatment. (After Johnson et al., 4)



oil from coal gasification wastwaters are necessary for evaluating treatment methods. This section describes the results of a study directed at the removal of tar and oil from a representative wastewater produced during coal gasification operations. The control methods investigated include coagulation using alum and synthetic organic polymers and acidification to precipitate dissolved tars and oils.

EXPERIMENTAL METHODS

Sample Handling and Storage

A five-gallon sample of a representative coal gasification wastewater was obtained from the Industrial Environmental Research Laboratory (IERL) of the U. S. Environmental Protection Agency (EPA) at Research Triangle Park, North Carolina. (For the pretreatment studies directed at tar and oil and grease removal, it was decided to work with real coal conversion wastewaters.) The sample was received in September, 1977 and was immediately stored in a $10^{o}C$ refrigerator. In an effort to maintain sample integrity, no attempt was made to preserve chemically the wastewater. The only major changes observed in this wastewater over the study period were a gradual darkening of the amber color and some settling of suspended materials. The wastewater was always well mixed prior to withdrawing samples in order to ensure that representative samples would be collected. The last few samples taken from the container were somewhat concentrated with respect to solids, however.

Experimental Procedures

Initially, the sample was characterized with respect to pH, TOC, COD, total phenols, tar, oil and grease, and suspended solids. Standard jar test procedures were then employed to investigate the effects of coagulation and precipitation on the sample. The procedure for each set of jar test experiments is outlined below.

Procedure for Coagulation Experiments--

A series of 100 ml samples of well-mixed wastewater were removed from the refrigerated storage container with a volumetric pipet. Each sample was placed in a 150-ml beaker and covered with Parafilm. The aliquots were allowed to warm to room temperature, usually 18° to 22°C, before experimentation began.

The pH of each sample was adjusted to the desired level by the dropwise addition of H_2SO_4. The pH was measured using a Fisher combination electrode and pH meter.

Various dosages of coagulant were added. During the course of coagulant addition, the pH was maintained constant by adding NaOH, if necessary.

Samples were flash-mixed for 1 min on a magnetic stirrer to disperse the coagulant. Samples were removed from the stirrer and immediately placed on a Phipps and Bird jar test machine for 20 min, at a mix speed of 20 RPM. No attempt was made to prevent the loss of volatile materials such as NH_3, H_2S, CO_2, or volatile, low molecular weight organic species during the stages of mixing.

After mixing, the samples were removed from the jar test machine and allowed to settle under quiescent conditions. After 1 hour, aliquots of

the supernatant were removed from each beaker by carefully lowering the tip of the pipet beneath the surface of the sample. Fifty-ml were removed for tar, and oil and grease analyses, and a 5 ml sample was collected for TOC, COD, and total phenols analyses. The solid residue remaining in the beaker after settling was filtered and analyzed for settleable solids.

Coagulants Used in Experiments--

Alum ($Al_2(SO_4)_3 \cdot 18H_2O$)--Stock solutions of 5, 20, and 50 g/l were prepared as needed from reagent grade alum. Use of a particular stock solution depended on the dose of alum required in the jar test experiment.

Organic Polyelectrolytes--DEAE-Dextran, a high molecular weight (2,000,000) cationic polyelectrolyte made from Dextran 2000, and Dow C-31 Purifloc, a cationic polyelectrolyte with a molecular weight of 30,000 were employed in these experiments. Stock solutions of 1 percent (10 g/l) Purifloc were made by pipetting 5 ml of the 20 percent concentrated stock solution and diluting to 100 ml. Stock solutions of 1 g/l of the dry DEAE-Dextran powder were made by slowly adding the weighed amount of polymer to distilled water and mixing in a high-speed Waring blender. Once dissolved, the solution was removed from the blender and diluted to 1 l. A stock solution of 0.1 g/l was made by dilution of the 1-g/l stock.

Procedure for Acidification Experiments--

Experiments designed to determine the effectiveness of acid addition on tar removal were conducted using procedures similar to those of the coagulation experiments. However, no coagulants were added after the pH was adjusted to the desired level. Thereafter, the samples were mixed and

allowed to settle for 1 hour, and aliquots of the resultant supernatant layer were withdrawn for analysis as in the coagulation experiments.

Analytical Methods--

Total Organic Carbon (TOC)--TOC was used as an indicator of the degree of removal of organic material from the wastewater samples by determining values before and after treatment. TOC was measured using a Beckman 915A TOC Analyzer coupled with a Beckman 215B Infrared Detector. Organic and inorganic carbon standards were prepared with carbon-free water according to methods outlined by Beckman Instruments; 1,000 mg/l stock solutions of the organic standard (reagent grade potassium acid phthalate) and the inorganic standard (a solution of $NaHCO_3$ and Na_2CO_3) were prepared. All other standards were subsequently made from dilutions of the 1,000 mg/l stocks.

Duplicate 10- or 20-µl injections of the organic and inorganic standards were made into the total carbon and inorganic carbon channels, respectively. Calibration curves were obtained by plotting percent absorbance vs. mg/l of total carbon or inorganic carbon.

Since there was concern that the wastewater may contain significant concentrations of volatile organics, the samples were not acidified and purged with nitrogen to remove inorganic carbon prior to analysis as recommended in Standard Methods (8). Instead, separate determinations of inorganic carbon were made as described in the following paragraph.

Duplicate 10- or 20-µl injections of each sample were made with an automatic syringe to ensure reproducibility. In cases for which the resulting peak heights were not consistent for the same sample, injections

were made until reproducible peak heights were obtained. Because the TOC of the wastewater was very high, dilutions of the treated and untreated samples were made in order to obtain values that would be "on scale" (between 0 and 1,000 mg/l). Typically, 5 ml of sample was diluted to 100 ml using distilled, deionized water. Absorbance readings on successive injections were averaged, and total carbon and inorganic carbon values were determined from the calibration curves. Inorganic carbon values were then subtracted from the total carbon values, yielding TOC values.

Acetone-Soluble Tar; Oil and Grease--Tar is operationally defined as an acid-insoluble organic residue. Its meaning is derived strictly from the procedure employed in its measurement. This analytical procedure was suggested by Johnson et al. (4) and modified for use during this study.

Oil and grease, operationally defined as acid-insoluble, freon-soluble material, was determined in accordance with the partition-gravimetric method outlined in Standard Methods , Section 502A (8).

Oil and grease, and tar determinations were usually made on the same aliquot. For the raw wastewater, a 50 ml aliquot of a well-mixed sample was removed from the storage container. For treated samples, including controls, 50 ml was withdrawn from the supernatant layer after chemical treatment and settling of solids. These aliquots were placed in 125 ml separatory funnels. The pH in all cases was then depressed to 1.6 by the dropwise addition of H_2SO_4 to precipitate all acid-insoluble materials. These acid-insoluble materials were then extracted with three 15-ml washings of freon, 1,1,2-trichloro-1,2,2-trifluorethane (reagent grade from Fisher Chemical Company). The three washings were combined in another separatory funnel where any water extracted into the freon was

allowed to separate from the freon. The freon layer was then poured into a clean, dry distilling flask of known tare weight. The freon was driven off under an applied vacuum through a rotary evaporator over a 70°C water bath. The flask was immediately transferred to a dessicator for 30 min and then weighed on an analytical balance. The difference in weight between the tare weight and the weight of the flask plus the freon-extracted material equaled the weight of oil and grease in the 50-ml aliquot of the supernatant. This value was subsequently multiplied by 20 to determine the concentration of oil and grease in mg/l.

The acid-insoluble residue remaining in the supernatant aliquot after freon extraction was drained onto a #40 Whatman filter disk to which a vacuum was applied. The filter disk was then washed with reagent grade acetone until it was apparent from the color of the disk that all acetone-soluble material had been extracted. The amount of acetone-soluble residue was a very small fraction of the total residue.

These acetone-soluble washings were collected in a 250 ml beaker and subsequently refiltered through another #40 Whatman filter disk to remove any acetone-insoluble material that might have been rinsed off the original filter disk. Following filtration, the acetone solution was poured into a clean dry distilling flask of known tare weight and rotary evaporated at 70°C, until all the acetone was removed. The flasks were placed in a 103°C oven for 30 minutes to remove any water contamination. The flask was removed from the oven, cooled to room temperature in a dessicator, and weighed on an analytical balance. The difference between the weight of the flask and the acetone-extracted residue and the tare weight of the flask equaled the weight of acetone-soluble tar in the 50 ml aliquot of

supernatant. As with oil and grease, this value was multiplied by 20 to obtain the concentration of tar in mg/l.

On some supernatant samples, only acetone-soluble tar was determined. The pH of these samples was depressed to 1.6 as before. After the solids precipitated, they were filtered through a #40 Whatman filter disk, and the freon extraction step was omitted. Thereafter, the procedure was identical to that outlined above for tar determination.

Chemical Oxygen Demand (COD)--COD was determined for selected samples according to the procedures outlined in Section 508 of Standard Methods (8). Samples were diluted in the same way as with TOC.

Total Phenols--The concentration of total phenols was determined by the 4-aminoantipyrine colorimeteric procedure outlined in Standard Methods, Section 510 A and C (8, 9). Phenols react with 4-aminoantipyrine at pH 10.0 $\pm$0.2 in the presence of potassium ferricyanide to form a colored antipyrine dye. Distillation of the sample is required as a first step to separate the phenolic compounds from nonvolatile impurities that interfere with color formation. The phenolic compounds measurable by this technique include phenol (C_6H_5OH), ortho- and meta-substituted phenols, and under certain pH conditions, para-substituted phenols in which the substituent is a carboxyl, methoxyl, halogen, or sulfonic acid group. All other para-substituted phenols cannot be detected by this procedure.

Samples had to be diluted so that no more than 0.5 mg phenol was present in 100 ml. The standard curve was not linear for values above this concentration. After distillation of the sample and addition of the reagents, absorbance of the colored antipyrine dye was measured at 510 nm with a Varian spectrophotometer. These readings were compared with a

standard curve of absorbance vs. mg phenol/100 ml. Results are reported as mg/l C_6H_5OH.

Because the percentage of various phenolic compounds present in the given coal gasification wastewater was not known, phenol itself was selected as the standard. Any color produced by the reaction of other phenolic compounds with the reagents is reported as phenol (8). Substitution generally reduces color response, so the values reported for total phenols in the wastewater are less than they would be if an equivalent amount of phenol was present in the wastewater.

Settleable Solids--Settleable solids are operationally defined as the amount of nonfilterable residue created after wastewater samples are chemically treated and allowed to settle for 1 hour. After aliquots of the supernatant layer were withdrawn for TOC, COD, tar, and oil and grease analyses, the remaining layer of settled solids was filtered through a Whatman glass fiber filter and analyzed for nonfilterable residue in accord with Standard Methods, Section 208D (8).

RESULTS AND DISCUSSION

General Wastewater Characteristics

Physical and chemical characteristics of the coal gasification wastewater sample are given in Table 3. The values of the parameters can be compared with the corresponding values given for Synthane gasification byproduct water in Tables 1 and 2. The wastewaters produced during the Synthane gasification process and the IERL-EPA wastewater sample are

reasonably similar with respect to the amounts of acetone-soluble tar and oil and grease.

TABLE 3. PHYSICAL AND CHEMICAL CHARACTERISTICS OF COAL GASIFICATION WASTEWATER SAMPLE.
(All values except pH in mg/l.)

pH	8.3
TOC	5,740
Oil and grease	920
COD	17,400
Acetone-soluble tar	1,950
Suspended Solids	750
Total Phenols	2,000

Treatment by Acidification

Figure 2 shows a plot of settleable solids created as a function of pH adjustment. Upon acidification, solids precipitate from solution and settle out under quiescent conditions. Solids production increases as acid is added, until a pH of about 5.7 is attained. Thereafter, no additional settleable solids are produced regardless of the amount of additional acid introduced to the system. Approximately 1,600 mg/l of settleable solids is produced by depressing the pH to any value below 5.7.

Figure 3 is a plot of the residual acetone-soluble tar as a function of pH. A gradual decrease in residual tar occurs as the pH is depressed from

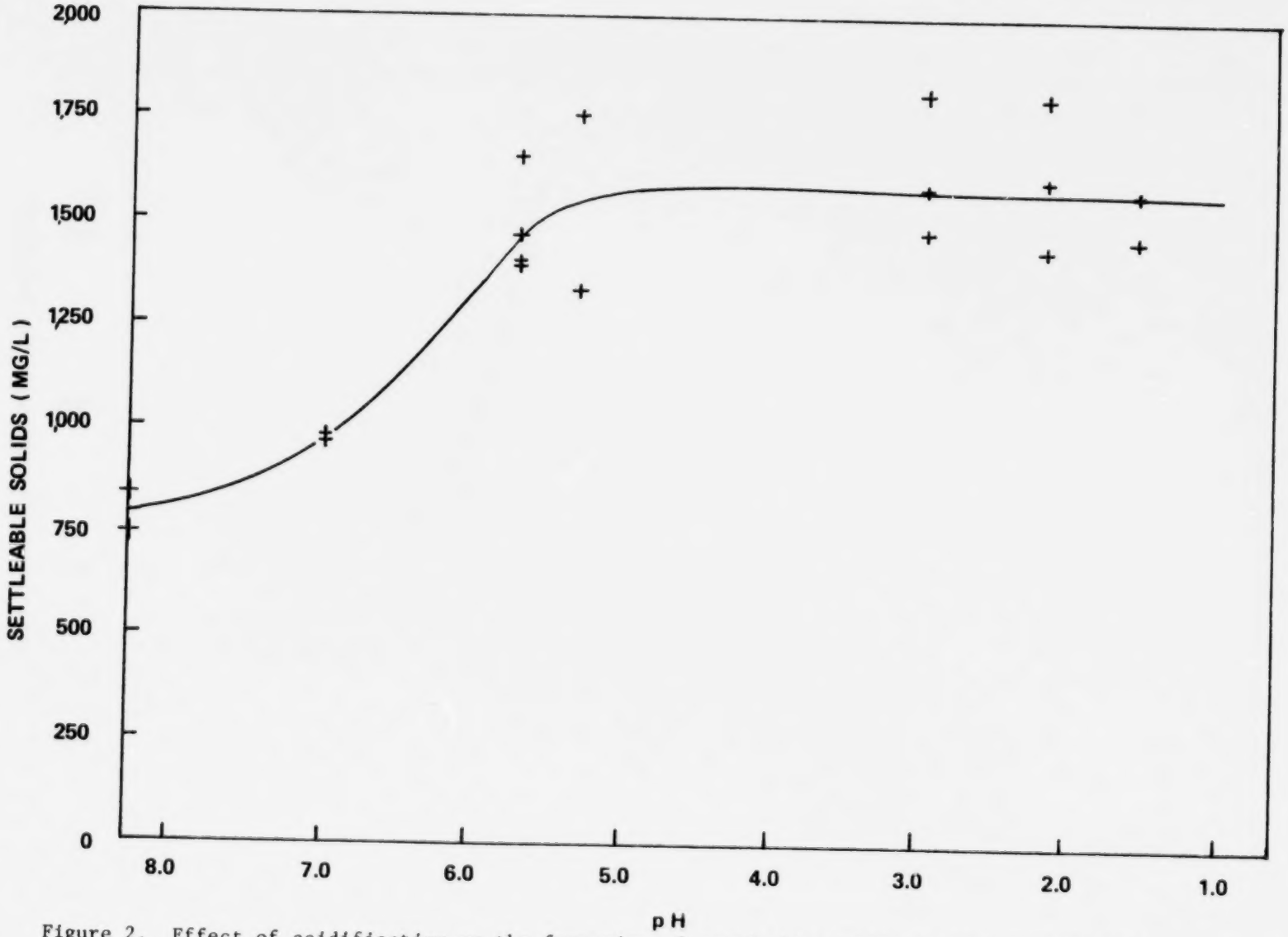


Figure 2. Effect of acidification on the formation of settleable solids in coal gasification wastewater.

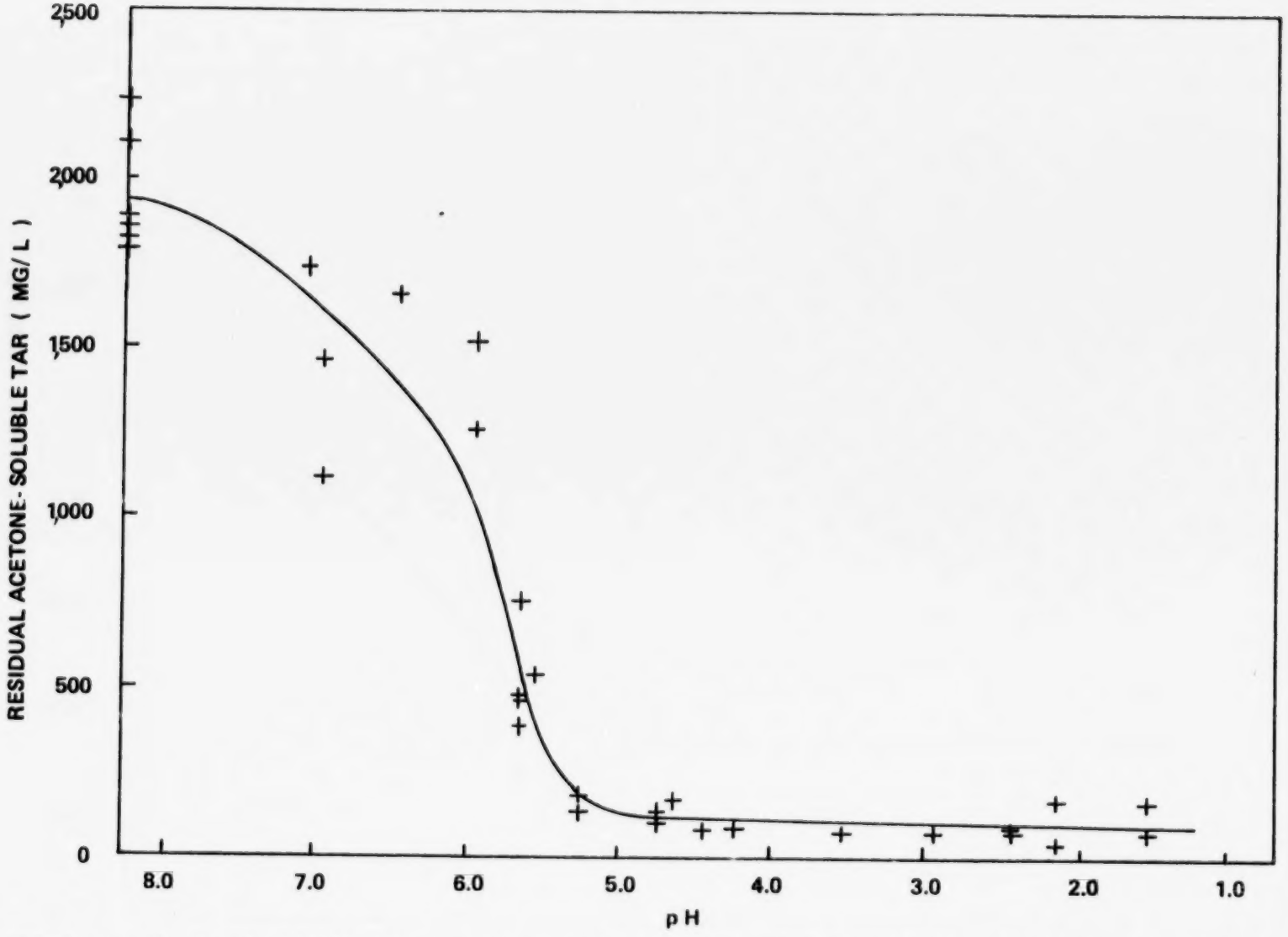


Figure 3. Effect of acidification on the removal of acetone-soluble tar from coal gasification wastewater.

8.3 to about 6.0. From pH 6.0 to pH 5.3, a sharp decrease in the concentration of residual tar is observed. Below pH 5.3, no significant improvement in tar removal is noted.

Some degree of scatter occurs among the data points, especially along the steep section of the curve around pH 6, but not enough to negate the observation of a trend. The scatter probably results because of difficulty in weighing heavy objects (distilling flasks) to find milligram differences in weight.

The analytical procedure for the determination of oil and grease was not effective in demonstrating removal trends of these impurities as a function of pH due to contamination of the solvent extract by water. When the freon extraction procedure was performed, it appeared that some of the amber-colored aqueous layer was miscible with the normally clear freon layer. The addition of an extra step to allow for separation of the water and freon layers did not eliminate the problem. Once the freon was evaporated, small amber-colored water droplets remained in the distilling flask. The flask was weighed, and the values obtained were reported as acid-insoluble, freon-soluble oil and grease. When freon was added to the distilling flask in order to dissolve the oil and grease for cleaning purposes, it was noted that all of the so-called oil and grease did not dissolve. It was evident that complete separation of true oil and grease from treated and untreated wastewater was not possible because of contamination of the oil and grease extract by water. The oil and grease analysis adopted for these experiments (8) was designed for the petroleum and food processing industries. Its applicability to the analysis of this coal gasification wastewater is questionable.

Figures 4 and 5 show TOC and COD removal as a function of pH. The greatest increase in removal of each is observed between pH 6.5 and 4.8. The actual shapes of the curves correspond closely with each other and with those describing settleable solids production and acetone-soluble tar removal. The greatest increase in solids production and the greatest decrease in residual tar, TOC, and COD occur between pH 6.5 and pH 4.8.

A summary of the results of acidification of the wastewater to pH 4.8 is shown in Table 4. While approximately 1,835 mg/l of tar is removed from the wastewater by acidifying to pH 4.8, 1,550 mg/l of settleable solids is concurrently produced. In addition, TOC and COD reductions of 1,220 mg/l and 2,730 mg/l, respectively, are associated with the observed tar removal. In view of the aromatic nature of the molecules comprising the tar, these reductions in TOC and COD are consistent with each other and with the observed removal of tar.

Figure 6 depicts an acid-base titration curve describing the neutralization of the coal gasification wastewater by strong acid. Titratable species such as bicarbonate, ammonia, and carbonate are at very high concentrations in coal gasification wastewaters (2). Predictions concerning the quantity of strong acid required to precipitate tars can be made from the data described by the curve. It appears that approximately 40 meq/l of acid is required to depress the pH of the wastewater to about 5.0. At this pH, 95 percent tar removal can be achieved (refer to Figure 3).

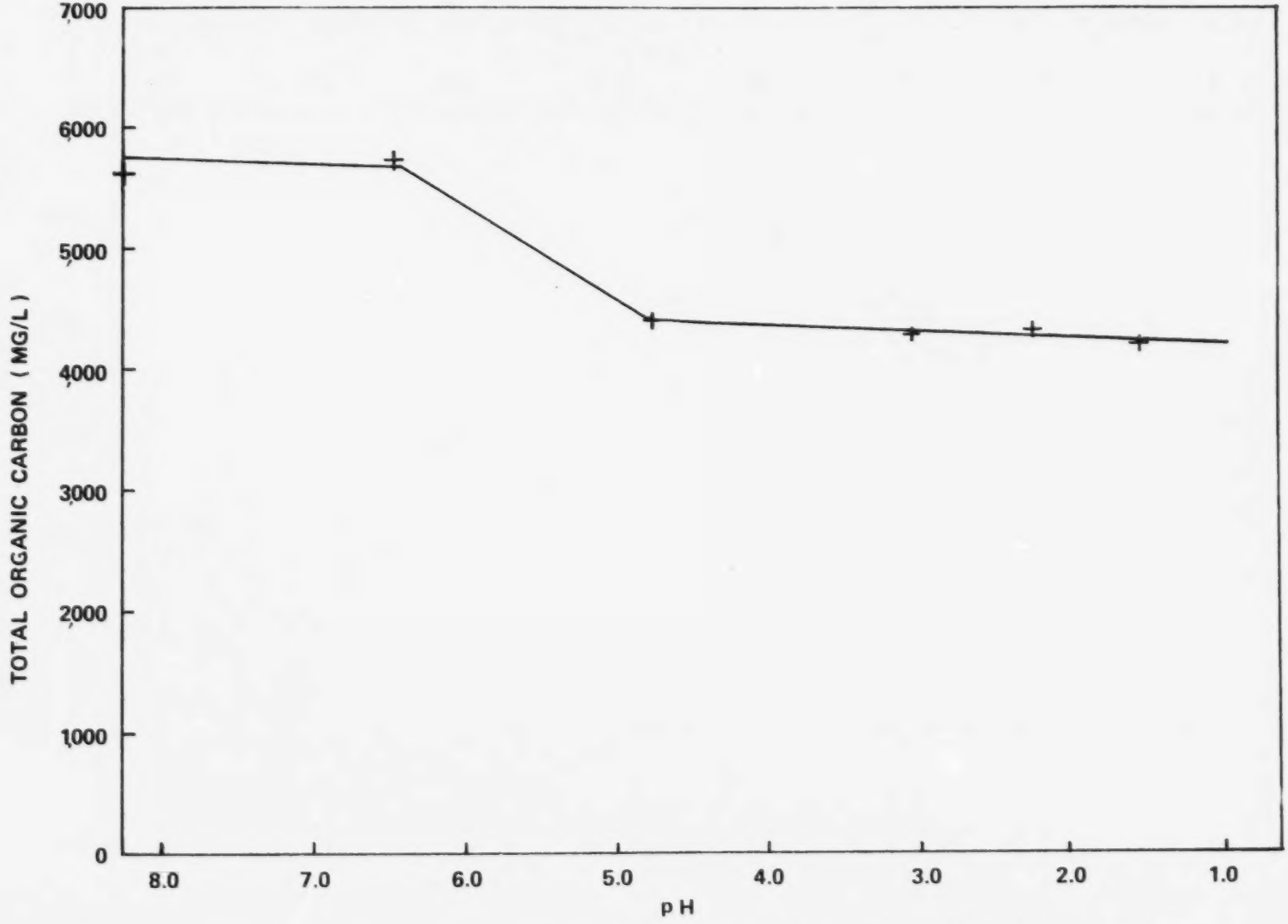


Figure 4. Effect of acidification on TOC removal from coal gasification wastewater.

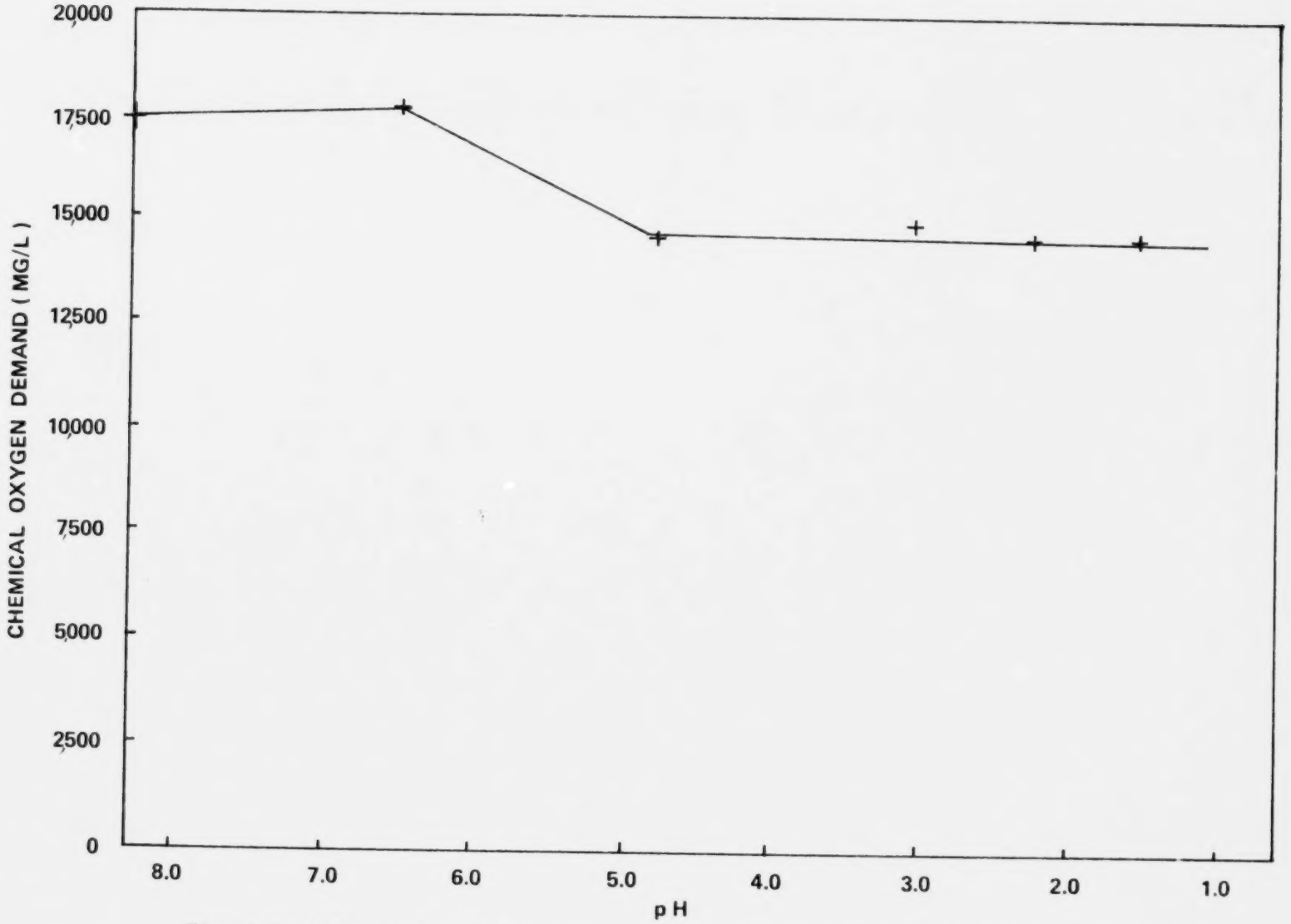


Figure 5. Effect of acidification on COD removal from coal gasification wastewater.

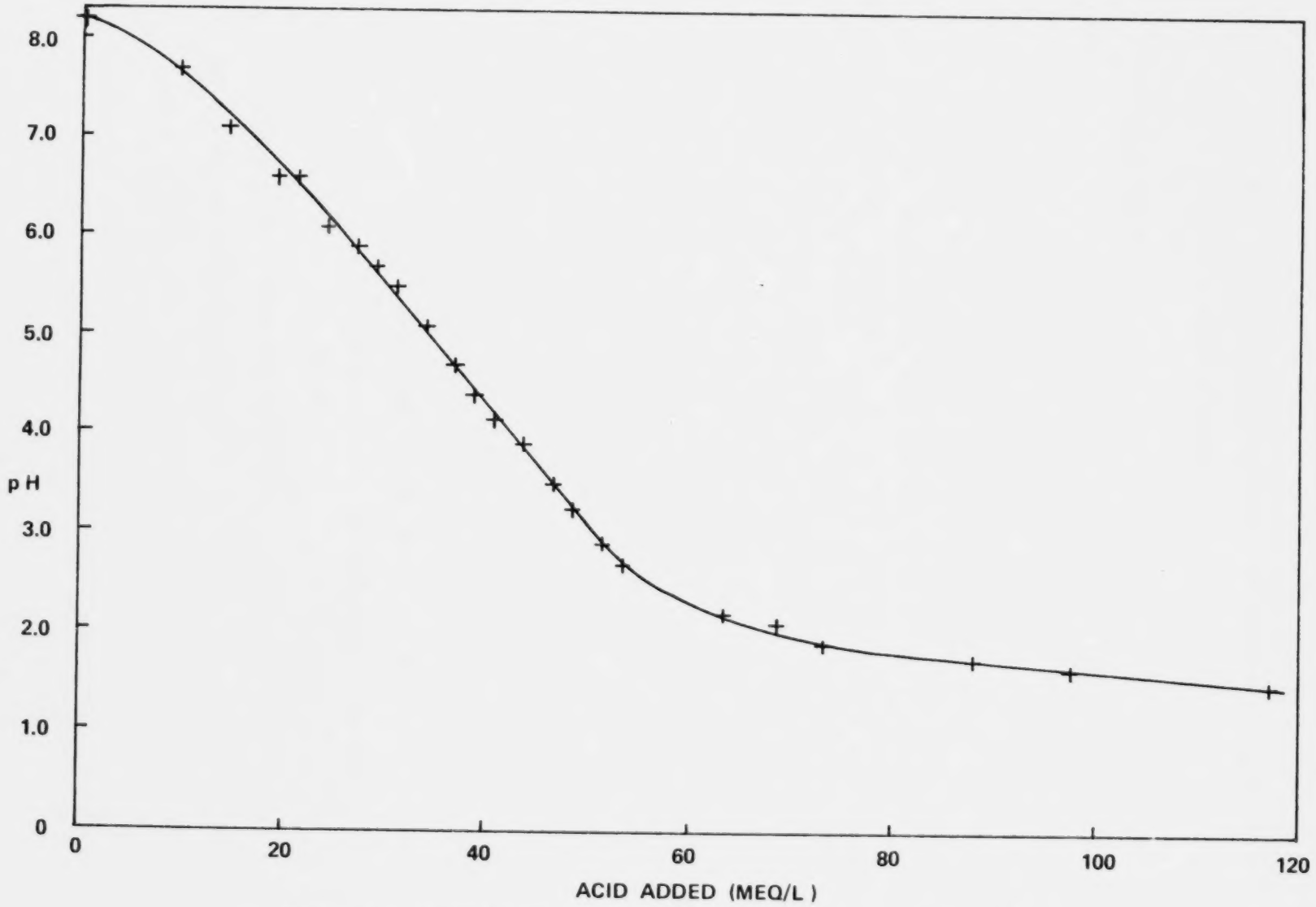


Figure 6. Titration of coal gasification wastewater with sulfuric acid.

TABLE 4. RESULTS OF ANALYSIS OF RESIDUAL SUPERNATANT AFTER ACIDIFICATION OF WASTEWATER SAMPLE TO pH 4.8

	Tar, mg/l	COD, mg/l	TOC, mg/l
Untreated	1,950*	17,400	5,620
Treated (acidified to pH 4.8)	115	14,670	4,400
Difference	1,835	2,730	1,220
% Reduction	94%	16%	22%

*Average of 6 determinations

Effectiveness of Coagulants

Alum--Figure 7 shows a plot of residual supernatant tar as a function of alum dose, at pH 5.7. As alum dosage is increased up to 1,000 mg/l, there is no concommitant reduction in acetone-soluble tar. In fact, there appears to be an increase in the tar concentration. The reason for this is unclear but could be due to restabilization of tar by the added Al(III). Initially, 1,950 mg/l of tar was present in the wastewater sample at pH 8.3. Tar is reduced to approximately 500 mg/l simply by acidification to 5.7.

The data presented in Figures 8 and 9 show that even at very high alum doses and at various pH values, little if any TOC removal is observed. The curve from Figure 5, which demonstrates TOC removal as a function of the

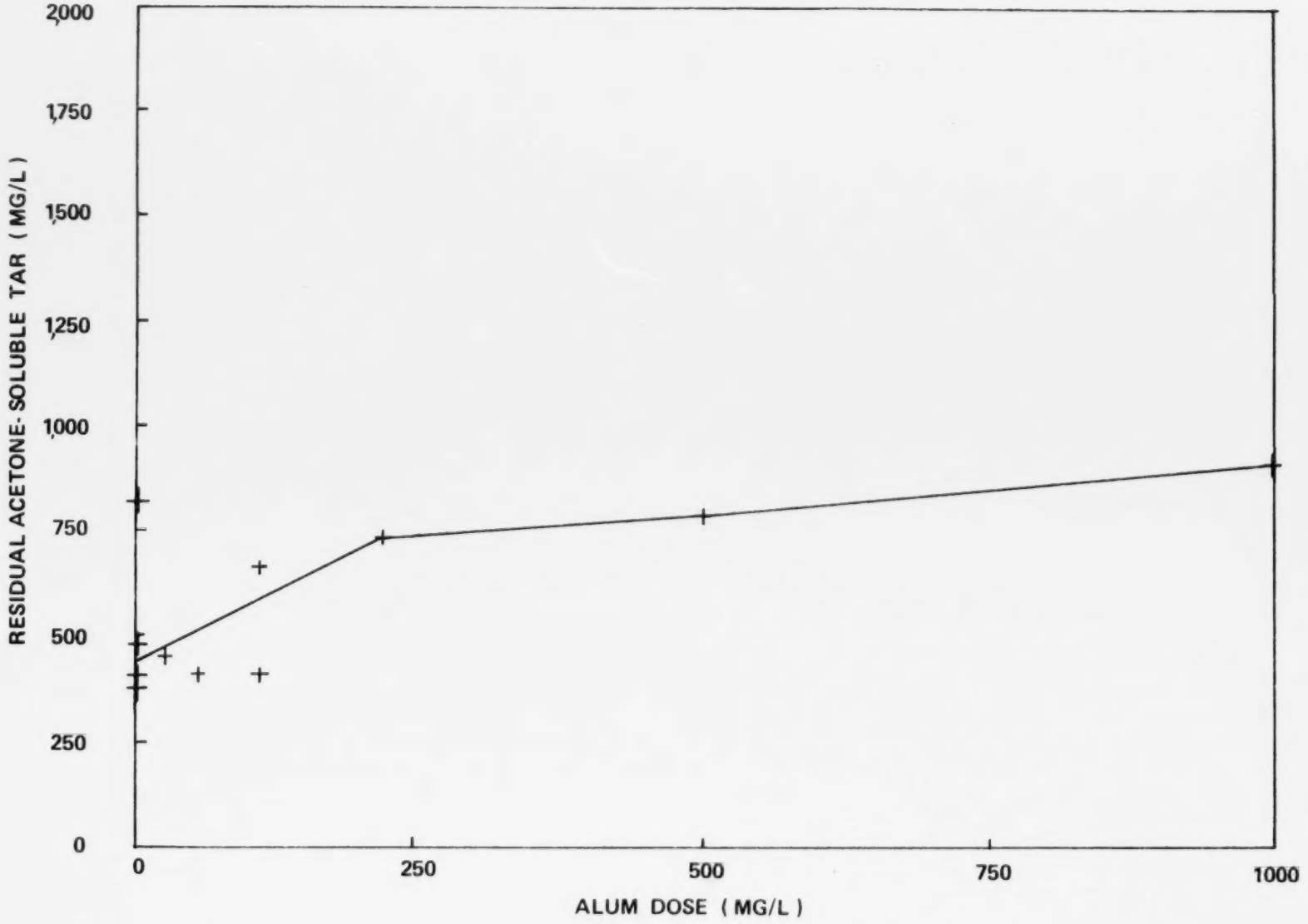


Figure 7. Removal of acetone-soluble tar by coagulation with alum at pH 5.7.

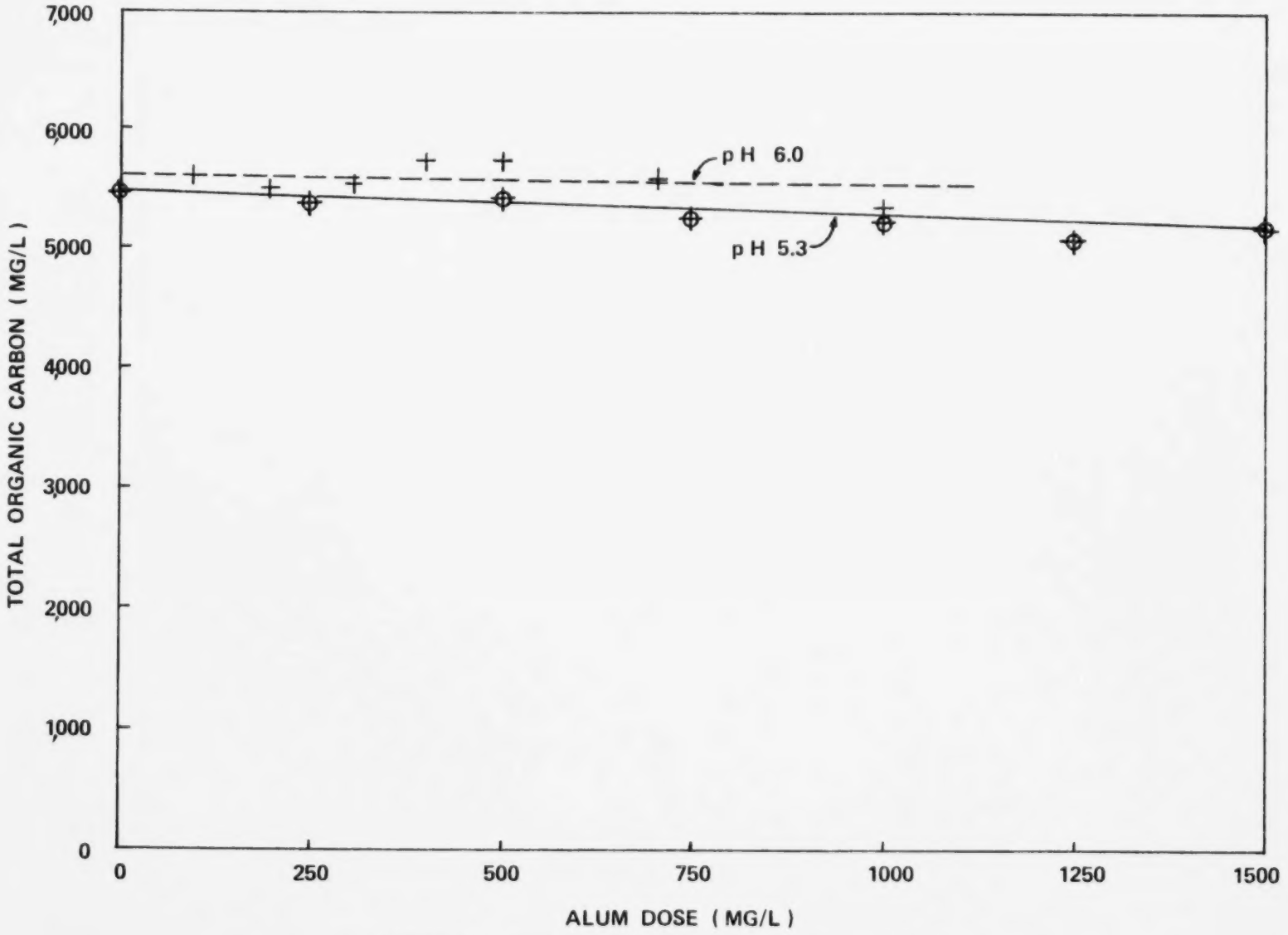


Figure 8. Removal of TOC by coagulation with alum.

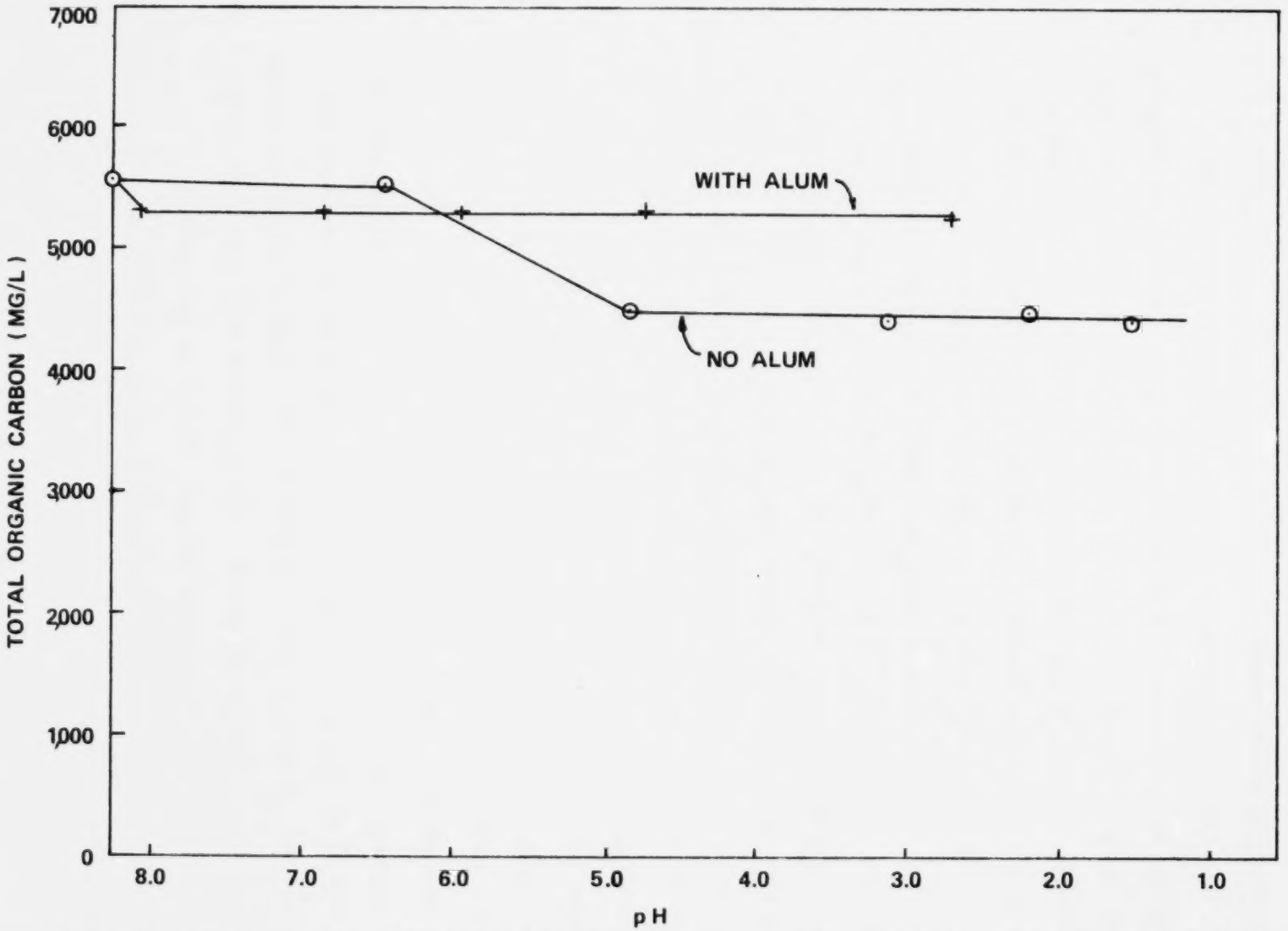


Figure 9. TOC removal by acidification in the presence and absence of alum. (Alum Dose = 1,000 mg/l.)

degree of acidification alone, without alum addition, is shown in Figure 9 for purposes of comparison.

From the data presented in Figure 10, it appears that the added aluminum is being totally solubilized by the constituents of the wastewater and that no precipitation of $Al(OH)_3$ is occurring. Normally, at pH 5.7, aluminum is insoluble and can be expected to hydrolyze to form abundant amounts of $Al(OH)_3$, which should precipitate from solution. Figure 10 demonstrates that no additional solids were created despite the massive addition of alum. This observation suggests that aluminum is being complexed (solubilized) by various dissolved species present in the wastewater and, as such, cannot be expected to act as a very effective coagulant as demonstrated in Figures 7, 8, and 9.

The presence of ligands with the potential of forming complexes with multivalent cations such as Al^{+3} is well documented for coal gasification wastewaters (2). Ligands capable of forming complexes with the Al^{+3} cation include CN^-, SCN^-, and the anions of organic acids, all of which can compete with hydrolysis reactions. In view of the alum dosage applied to the samples (up to 1,500 mg/l as alum), the ability of the ligands in the wastewater to complex aluminum must be considerable.

Polymers--In contrast to alum, the two cationic polyelectrolytes tested in this study were shown to be capable of removing tar and TOC from the wastewater. Figures 11 and 12 demonstrate the effect of increasing dosages of DEAE-Dextran applied at pH 6.0. Tar is reduced from 1,260 mg/l to 260 mg/l at pH 6.0 upon addition of 100 mg/l of DEAE-Dextran. TOC is concommitantly reduced by 300 mg/l at this dosage and by greater amounts at higher polymer dosages.

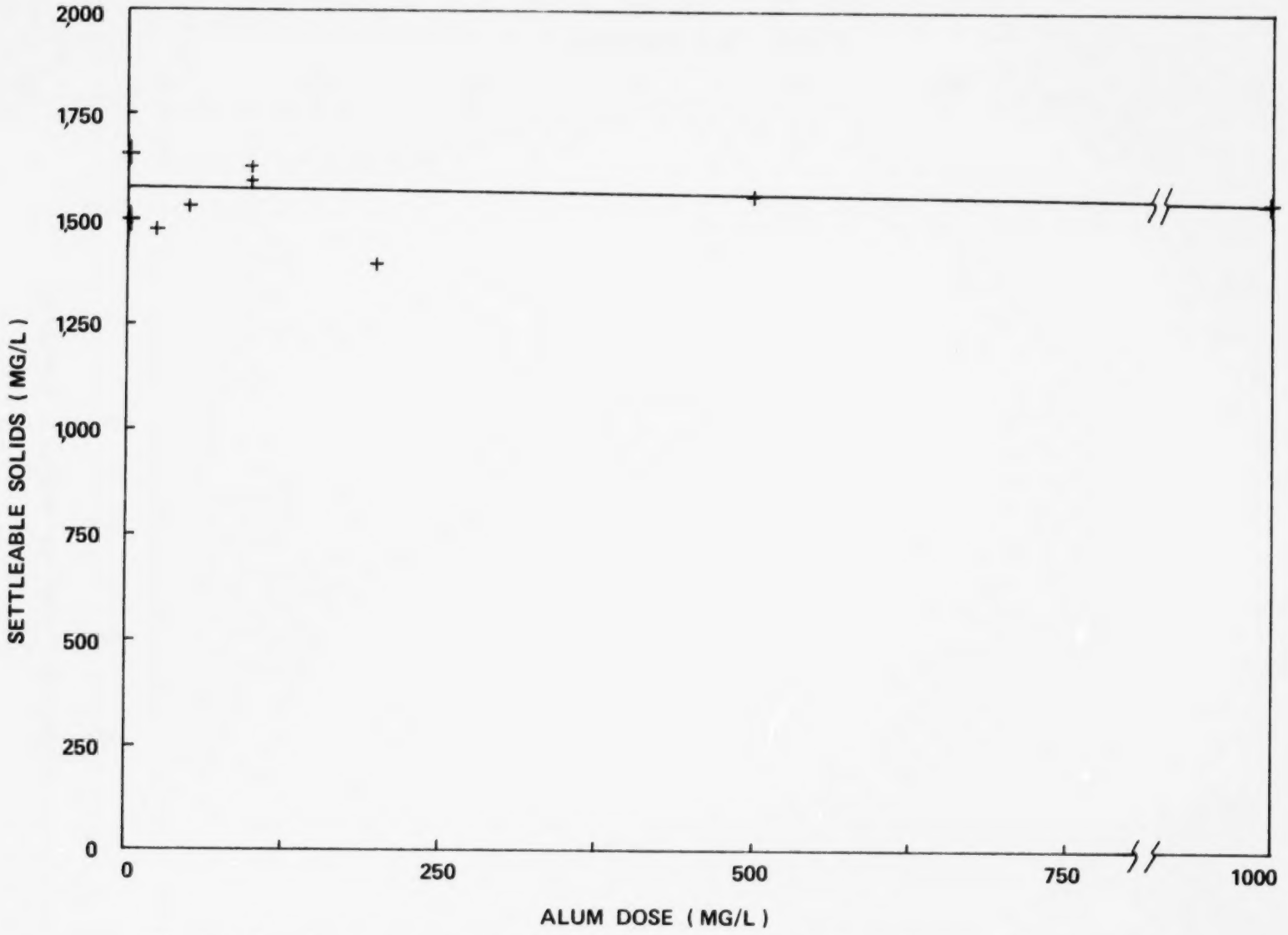


Figure 10. Production of settleable solids resulting from alum coagulation at pH 5.7.

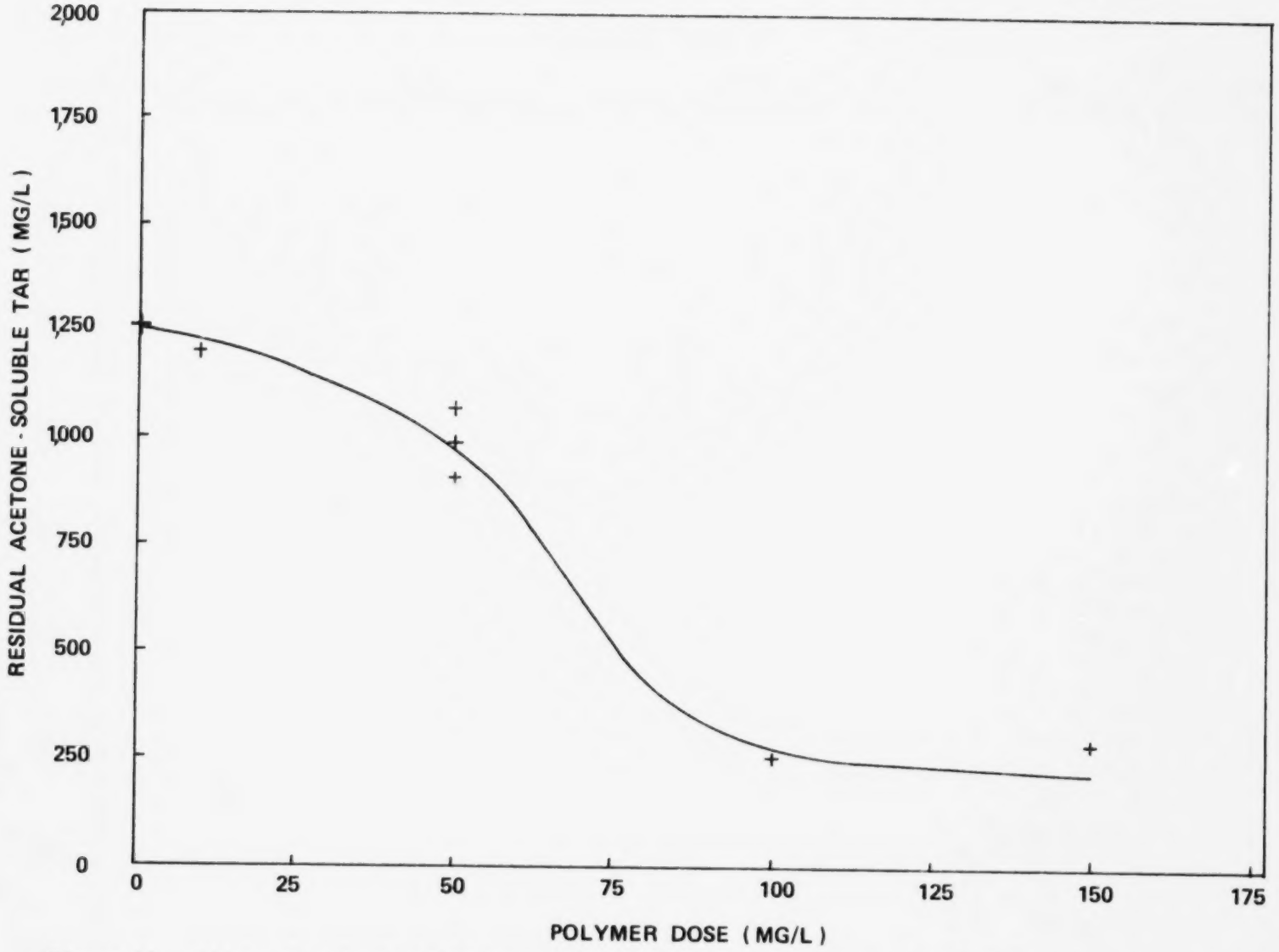


Figure 11. Effect of polymer (DEAE-Dextran) addition on the removal of acetone-soluble tar at pH 6.0.

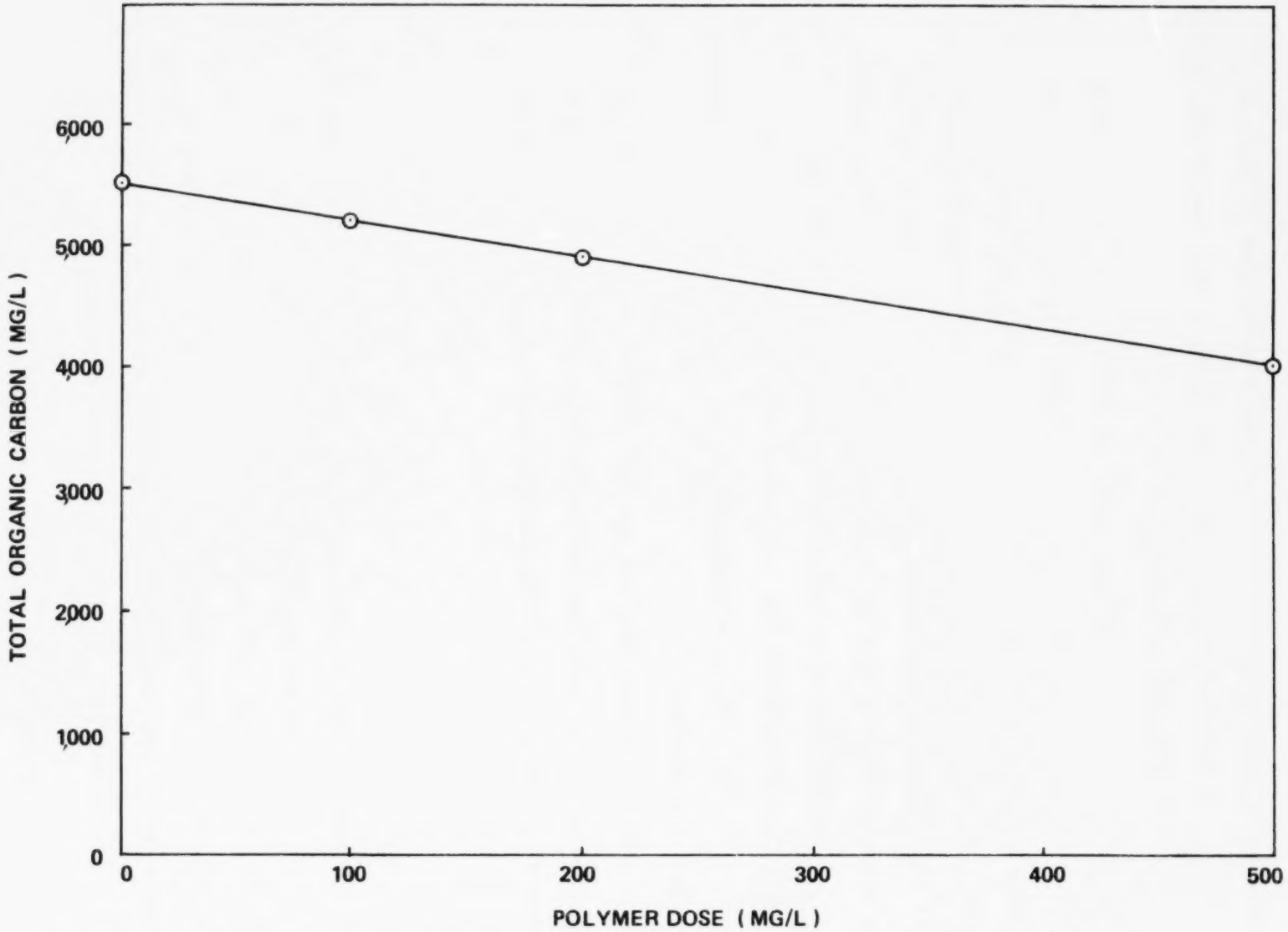


Figure 12. Effect of polymer (DEAE-Destran) addition on the removal of TOC at pH 6.0.

Similarly, Dow C-31 Purifloc was also capable of removing TOC from the wastewater. A TOC reduction of 600 mg/l was observed when 200 mg/l of Purifloc was applied at pH 8.3. No determinations of tar removal were made during these experiments, however.

DEAE-Dextran has a molecular weight of 2,000,000 (10) which is more than sufficient for destabilization by interparticle bridging. Purifloc does not have as high a molecular weight, but does have a higher charge density and can act to destabilize by adsorption and charge neutralization.

Considerable dosages of DEAE-Dextran and Purifloc had to be applied before significant reductions in tar and TOC were observed. Some polymers can destabilize certain colloidal suspensions at very low dosages (10), making them economically desirable to use. The dosages required to destabilize tar in these wastewaters appear to exceed dosages that would be economically feasible.

Further experimentation with Purifloc and DEAE-Dextran at different dosages and pH's and experimentation with other cationic, anionic, and nonionic polymers was precluded due to lack of a sufficient quantity of wastewater.

Total Phenols

The concentration of total phenols was determined for samples that were acidified as well as for samples that were acidified and treated with various doses of alum. The concentration of total phenols was not decreased by any type of treatment (Table 5). While the solubility of acetone-soluble tar is dramatically reduced as the pH is depressed, no change in the solubility of phenolic compounds is observed.

TABLE 5. PHENOL REMOVAL BY ACIDIFICATION AND COAGULATION.
(All values in mg/l.)

pH	Alum Dose mg/l)	Total Phenols (mg/l as phenol)
8.3	----	1,900
8.3	----	2,030
8.3	----	1,920
3.1	----	2,000
2.8	1,000	1,920
5.0	1,000	1,920
5.3	250	2,000
5.3	1,000	1,950

Summary

Results of acidification experiments on the coal gasification wastewater supplied by the Industrial Environmental Research Laboratory (IERL) showed that a 94 percent reduction in residual acetone-soluble tar could be obtained when the pH of the sample was depressed to approximately 4.8 (Table 4). This reduction in tar corresponds to a 16 percent decrease in COD and a 22 percent decrease in the TOC of the wastewater. These values appear to be consistent with each other.

Johnson et al. (4) were able to achieve a 47 percent reduction in tar, oil, and grease in Synthane by-product water after batch treatment consisting of pH depression and alum addition followed by pH adjustment to

7.0 (Figure 1). In addition, Johnson et al. observed "floc formation" at various pH's and alum dosages up to 1,500 mg/l. However, for the wastewater used in this study, alum was incapable of acting as an effective coagulant, most likely because of the solubilization of aluminum by ligands present in the wastewater.

CONCLUSIONS

1. Acidification of a representative sample of coal gasification wastewater obtained from the EPA proved to be an effective means to remove dissolved and suspended acetone-soluble tars. TOC and COD reductions were observed concommitantly with tar reductions. In order to obtain about 95 percent tar removal from the wastewater, approximately 40 meq/l of strong acid was required.The addition of this amount of acid to the wastewater depressed the pH of the waste to about 5.0.

2. Alum was ineffective in chemical treatment of the wastewater. It is proposed that complexes are formed between aluminum and ligands present in the wastewater, resulting in the solubilization of aluminum and the inhibition of its effectiveness as a coagulant.

3. Two organic cationic polyelectrolytes were shown to be effective coagulants, but only at high dosages. The cost of these polymers most likely precludes their use on a large scale.

4. The analytical method for the determination of oil and grease was not effective in demonstrating removal of these materials from the wastewater.

SECTION 4

BIOLOGICAL TREATMENT OF SYNTHETIC COAL CONVERSION WASTEWATERS: PART 1

Aerobic biological processes will most likely be the principal means of treating coal conversion wastewaters for the removal of phenols and the other organic impurities. In order to evaluate the biological treatability of a wastewater, biotreatability studies should be conducted using the specific wastewater for which the treatment is being developed. In this study, however, and at this time, it is not feasible to use actual wastewaters from coal conversion operations since coal conversion processes are still in the developmental stage and it is unlikely that a suitable, consistent, and representative wastewater could be obtained. Accordingly, a synthetic organic wastewater was formulated to provide a mixture of organic compounds, at known and reproducible concentrations, to be used in acclimatizing and maintaining microbial cultures for preliminary biotreatability studies. The synthetic wastewater was used to feed several bench-scale pilot reactors. In addition to generating acclimatized organisms for biodegradability studies (see Section 9), analysis of effluents from the reactors provides information on wastewater characteristics before and after various degrees of biological treatment. This section presents preliminary results of this biotreatability study.

FORMULATION OF SYNTHETIC COAL CONVERSION WASTEWATER

Several criteria were employed in choosing specific compounds to be included in the synthetic wastewater, and their concentrations. Because it was desired to use this waste as a means of developing an acclimatized culture of microorganisms, most of the compounds selected are known or thought to be biodegradable. However, not all of the identified constituents of coal conversion wastewaters can be utilized by microorganisms. Accordingly, some compounds presumed to be slowly degradable or non-degradable, as deduced from earlier biodegradation experiments (1), were also included (e.g., 2-indanol, indene, 2-methylquinoline, and 3,5-xylenol).

In formulating the composition of the synthetic wastewater, it was desired that concentrations of the various components should be similar to those encountered in real wastewaters. Accordingly, reference was made to a summary of the constituents identified in coal conversion wastewaters (1), and the range, midrange, and median concentrations were determined for each constituent and for each class of compounds (e.g., cresols, xylenols, heterocyclic N-compounds, etc.). From each class, one or more compounds were chosen based upon considerations of biodegradability and reported concentration. The specific compounds chosen were usually the compounds within each class which were reported at the highest concentrations in the real wastewaters. Often, if a class contained many components, or if differences in biodegradability among the components of a given class were anticipated, more than one chemical from that class was chosen. The concentration selected was the median value reported for that compound in the real wastewater, or the median of the class if only one compound from

that class was picked. When the concentration of a specific compound selected was not known, it was included in the synthetic wastewater at the median concentration for its class.

Table 6 presents the composition of the wastewater formulated in this manner. Twenty-eight organic components are included, as well as inorganic nutrients and pH-buffers. The synthetic wastewater represents all major classes of organics present in real wastewaters for which data are available, and virtually all specific organic compounds which have been reported to be present at high concentration. The total organic carbon (TOC) concentration of all the components is 4,636 mg/l.

DESCRIPTION OF PILOT UNITS

Four 25-liter biological reactors were constructed for use in the initial phases of the pilot program. (The number of reactors has since been increased to eight.) Each reactor consists of a 7-1/2 inch ID lucite tube, four feet long, fitted at the bottom with a stainless steel cone with a 45^{o} slope (Figure 13). Each reactor has overflow and sampling connections located at appropriate heights to retain the desired volume of contents in the reactor and to permit withdrawal of samples from desired elevations. The stainless steel cone is equipped with connections to permit draining of the unit, if desired, and nipples for introducing air and feed solution at the bottom of the cone.

A compressor, operating through a pressure regulator, supplies air to each reactor at a rate adequate to insure thorough mixing and maintenance of aerobic conditions in the mixed liquor at all times. The rate of air supply is controlled through use of rotameters and needle valves.

TABLE 6. COMPOSITION OF SYNTHETIC COAL CONVERSION WASTEWATER

Compound	Concentration, mg/l
1. Phenol	2,000
2. Resorcinol	1,000
3. Catechol	1,000
4. Acetic Acid	400
5. o-Cresol	400
6. p-Cresol	250
7. 3,4-Xylenol	250
8. 2,3-Xylenol	250
9. Pyridine	120
10. Benzoic Acid	100
11. 4-Ethylpyridine	100
12. 4-Methylcatechol	100
13. Acetophenone	50
14. 2-Indanol	50
15. Indene	50
16. Indole	50
17. 5-Methylresorcinol	50
18. 2-Naphthol	50
19. 2,3,5-Trimethylphenol	50
20. 2-Methylquinoline	40
21. 3,5-Xylenol	40
22. 3-Ethylphenol	30
23. Aniline	20
24. Hexanoic Acid	20
25. 1-Naphthol	20
26. Quinoline	10
27. Naphthalene	5
28. Anthracene	0.2
theoretical TOC =	4,636 mg/l
NH_4Cl (1,000 mg/l as N)	3,820
$MgSO_4 \cdot 7H_2O$	22.5
$CaCl_2$	27.5
FeNaEDTA	0.34
Phosphate Buffer: KH_2PO_4	170
K_2HPO_4	435
$Na_2HPO_4 \cdot 7H_2O$	668

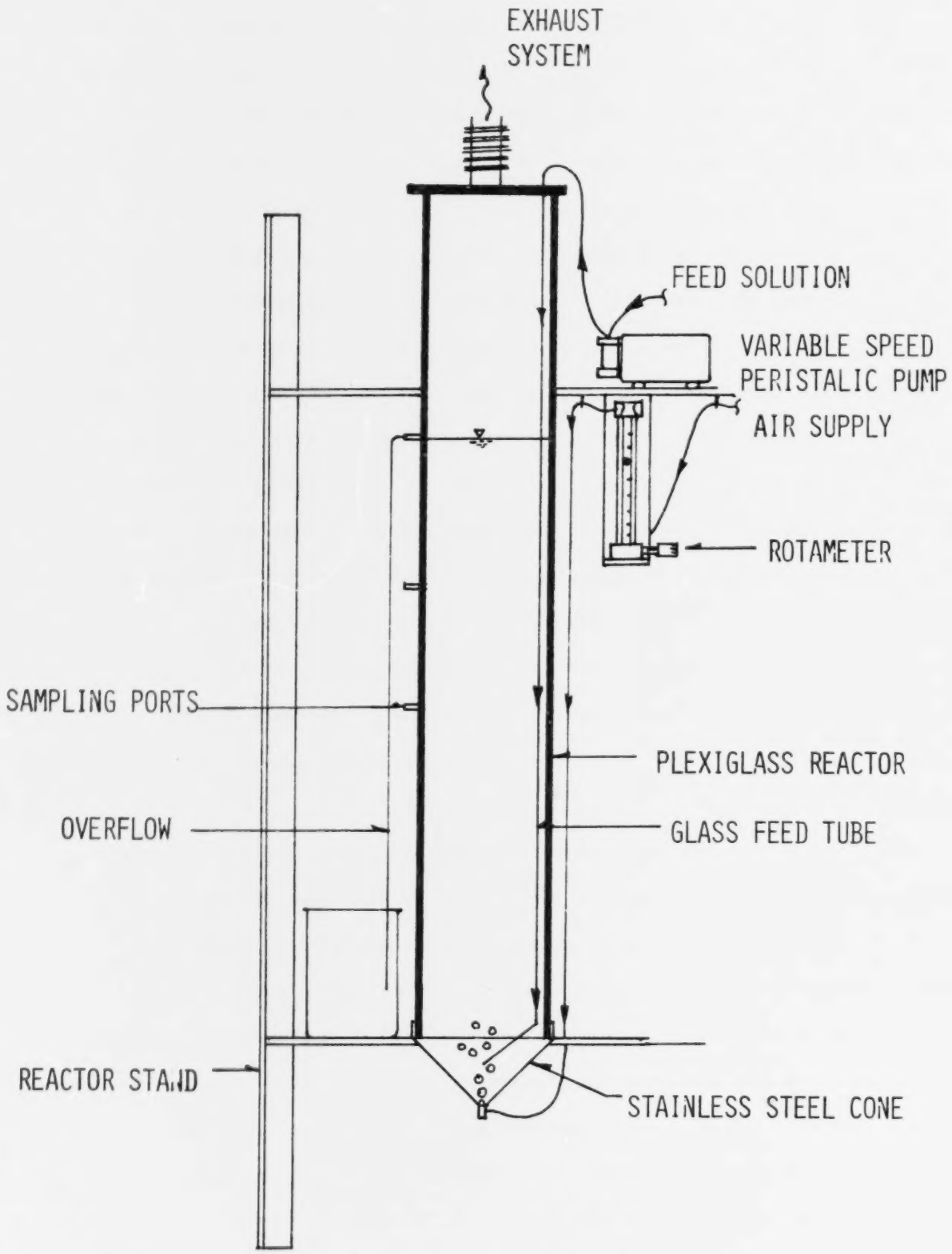


Figure 13. Diagram of experimental biological reactors.

The units were fed synthetic wastewater from a glass storage reservoir mounted on a large magnetic mixer. The wastewater was introduced into each reactor by a variable-speed peristaltic pump. During this phase of the biotreatability study, the reactors were operated as continuous-flow activated sludge systems with no recycle of solids (biomass). Hence, solids residence time (sludge age) equalled hydraulic detention time. Hydraulic detention times of 5, 10, and 20 days were investigated during this first phase of study. The pump feeding the 5-day reactor was operated continuously. Pumps supplying feed to the other reactors were actuated by a clock which operated them for a predetermined period once every half hour. Two parallel reactors were operated at a 20-day detention time: one reactor was isolated for use as a chemostat to provide seed organisms for parallel biodegradation investigations, while the other 20-day reactor was used with the 5- and 10-day reactors to provide operating data to characterize reactor performance as a function of solids residence time. Overflow from each reactor was collected in a glass reservoir and the amount of wastewater actually fed was determined daily by measuring the amount of effluent collected in that container.

Because of the potential hazards associated with some of the chemicals in the wastewater, and because it was desired to eliminate objectionable odors in the working area, an exhaust system was installed to vent the units continuously to the outside of the building. The exhaust system consisted of a blower mounted at the outside wall, thereby maintaining the air ducts under a slight vacuum to insure that gases from the reactors always flow into the exhaust system and not into the room. The feed reservoir was also vented to the exhaust system in order to prevent the escape of gases into the room from that unit, as well.

OPERATION OF PILOT UNITS

The synthetic wastewater was made up in 16-liter batches. Carbon-filtered Chapel Hill tap water was used as dilution water to which the 28 constituents, shown in Table 6, were added. This was accomplished by adding appropriate quantities from concentrated stock solutions, prepared periodically from reagent grade chemicals and stored under refrigeration until use. It was found that in order to prepare some of the concentrated solutions an organic solvent was required to maintain solubility of the component organics. Accordingly, acetone was employed for this purpose. While this introduced an extra constituent into the wastewater, it was believed that much of the acetone would be removed through air stripping in view of the long detention times in the reactors. Hence, the TOC concentration of the raw wastewater was actually somewhat higher than that shown in Table 6.

The reactors were started up using activated sludge from one of the Durham, North Carolina municipal wastewater treatment plants. The feed of synthetic wastewater was increased gradually over a period of several days to allow time for acclimatization of the microorganisms to the wastewater. However, during the first few weeks after startup, all of the units began to fail as evidenced by increased TOC concentrations in the effluents and decreased solids concentrations in the reactors. Failure occurred first in the five-day reactor, then in the ten- and twenty-day reactors. The exact reason for failure is unknown, but several possibilities have been considered. Operating procedures during the early stages of the investigation were uncertain and made it possible for the concentration of dissolved oxygen in the reactors to drop occasionally to zero. Also, the

pH decreased to low levels (approximately 4) and remained there for extended periods. Further there is a possibility that some wastewater constituents could have exerted a toxic effect on the microorganisms as concentrations of the constituents built up in the reactor during the period following startup. The pattern of failure, i.e. in order of increasing reactor detention time, is consistent with the latter hypothesis.

Because of the possibility of toxic effects, and a desire to stabilize operations as quickly as possible, it was decided to reduce the strength of the synthetic feed to one-quarter of that in Table 6 during these initial investigations. Other investigators (3, 4) have had to resort to similar dilution procedures in order to treat coal conversion wastewaters biologically. The resulting diluted version, with a theoretical TOC of 1,159 mg/l is not inconsistent when compared with biotreatability experiments being conducted by others. (The concentration of TOC measured in the feed averaged 1,600 mg/l over the course of the runs due to the addition of acetone to solubilize the organic constituents in the feed.) At a later date, the question of treating the synthetic wastewater at higher strengths will be addressed. Accordingly, the reactors were started up again using a synthetic wastewater diluted to one-quarter of the concentrations specified in Table 6.

It should be noted that there was a significant change in color of the synthetic feed solution over the several days during which it was used to feed the reactors. Attempts were made to determine possible changes in wastewater composition during this time through periodic measurements of TOC and chromatographic scans using high performance liquid chromatography (HPLC). Chemical changes accompanying the change in color from clear to brown appeared to be minimal.

Routine sampling of each reactor was performed three times a week. Parameters measured included temperature, pH, mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), sludge volume index (SVI), and total organic carbon (TOC). pH was measured potentiometrically. Mixed liquor suspended solids concentrations were determined using glass fiber filters in a Buchner funnel, followed by drying of the filter in an aluminum dish at 103°C for 24 hours. Filtrates from MLSS analyses were collected for total organic carbon determinations using a Beckman 915 Carbon Analyzer. Sludge volume index (SVI) was determined by allowing mixed liquor from the reactors to settle for thirty minutes in a one liter graduated cylinder and calculating the settled volume occupied by the MLSS (8).

Other samples were collected as desired for the measurement of biochemical oxygen demand (BOD), chemical oxygen demand (COD), and for more detailed analyses, including specific organic compounds using HPLC and GC/MS, aquatic bioassays, and assessment of health effects. BOD and COD analyses were conducted on samples from which suspended materials had been removed through glass fiber filtration. Samples for HPLC and GC/MS analysis and for aquatic bioassay and health effects assessment were centrifuged, filtered, and frozen. The results of these analyses are presented in Section 7.

PRELIMINARY RESULTS

Figures 14 through 17 show performance characteristics for each reactor over the period from May to October 1978. The reactors operated without serious incident from the beginning of May to the middle of June. The

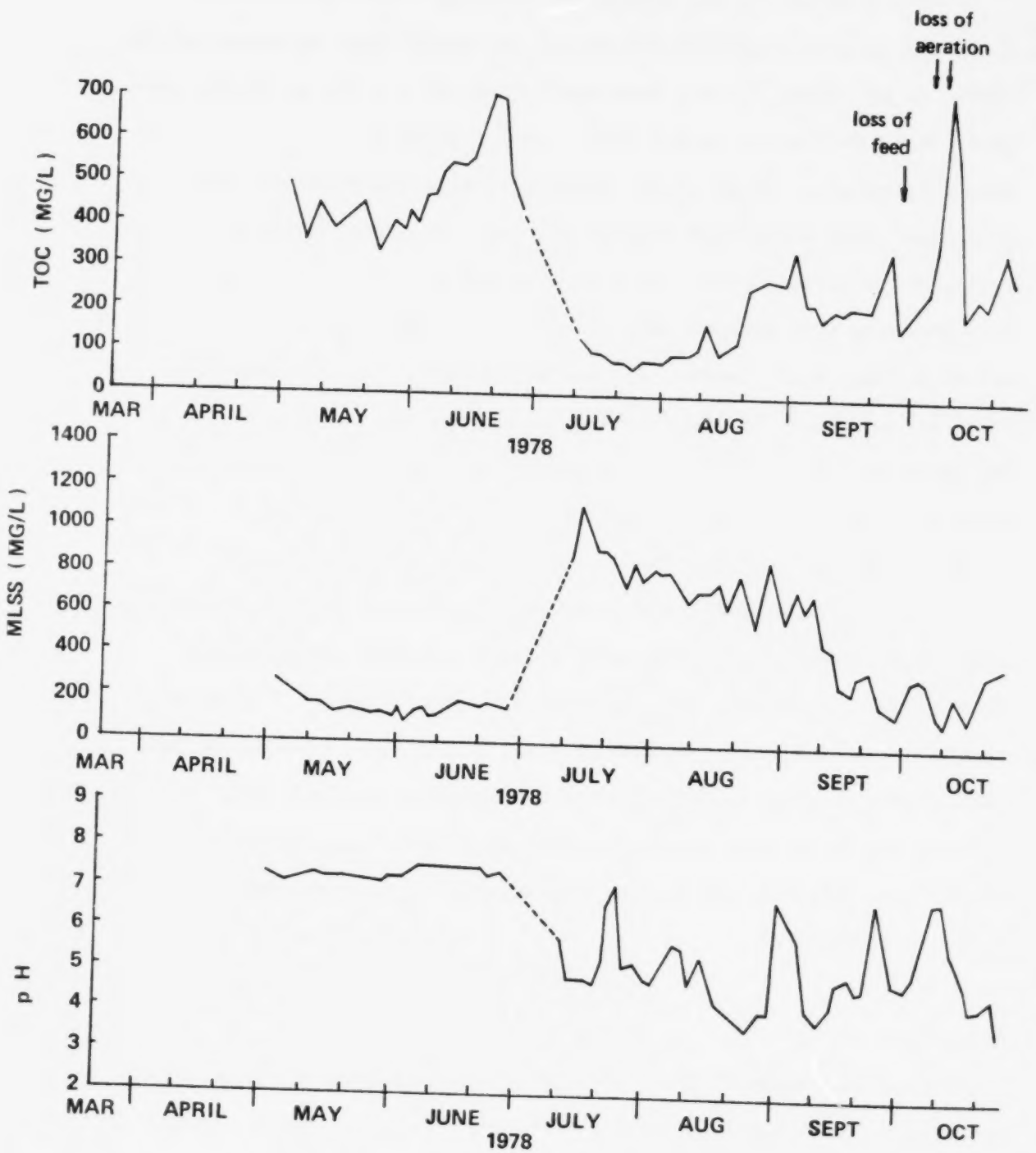


Figure 14. Performance characteristics of the 5-day reactor.

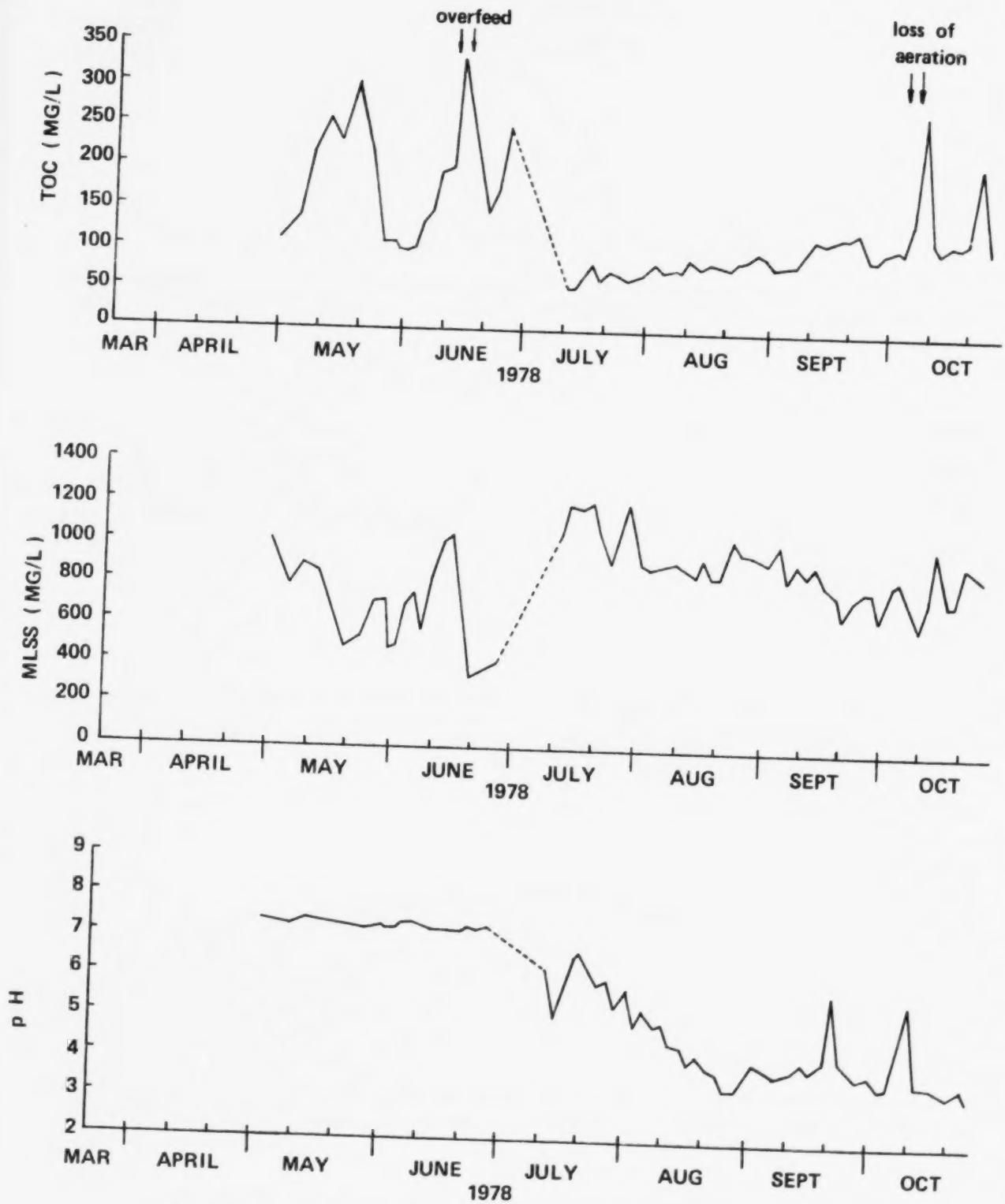


Figure 15. Performance characteristics of the 10-day reactor.

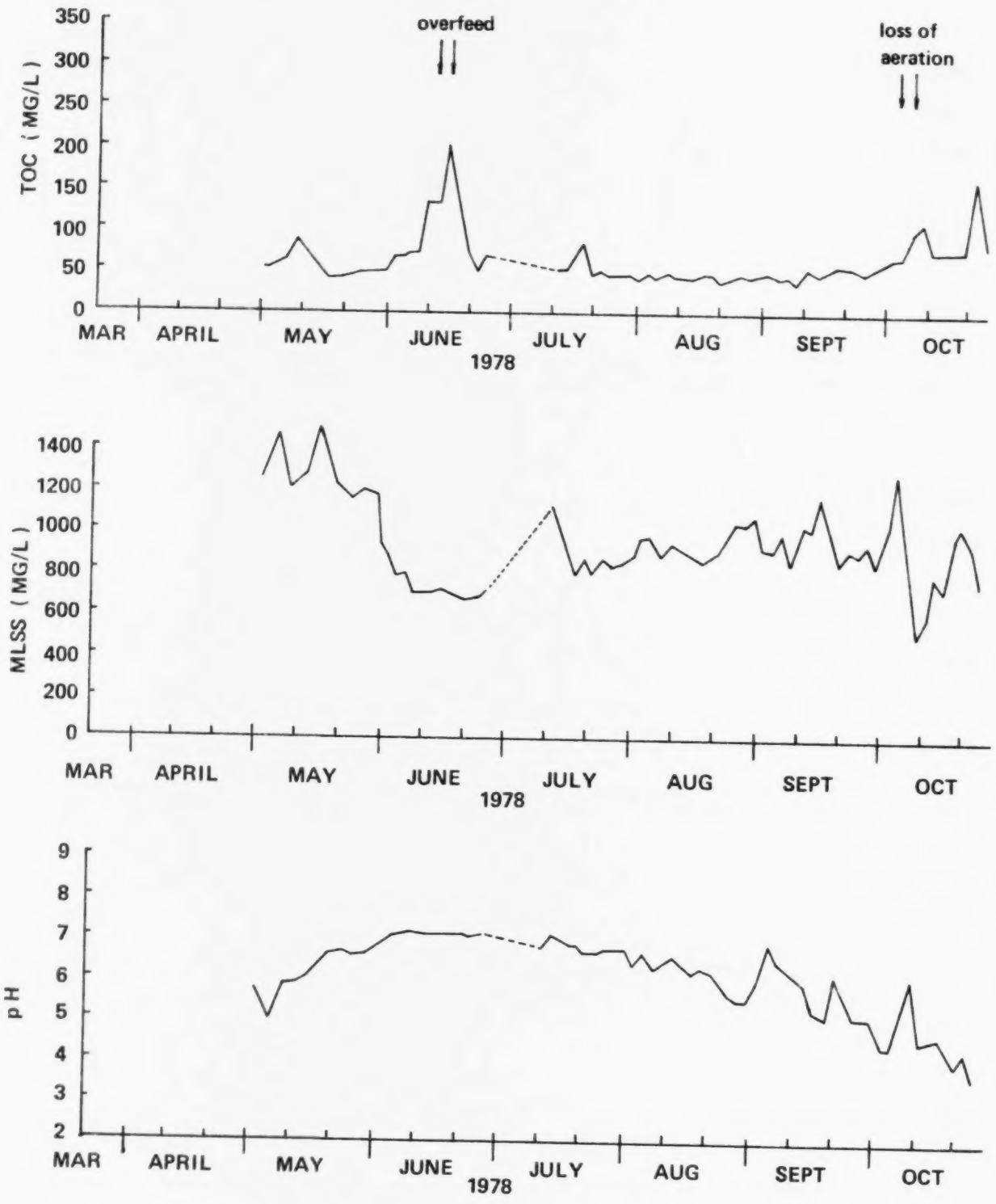


Figure 16. Performance characteristics of the first 20-day reactor.

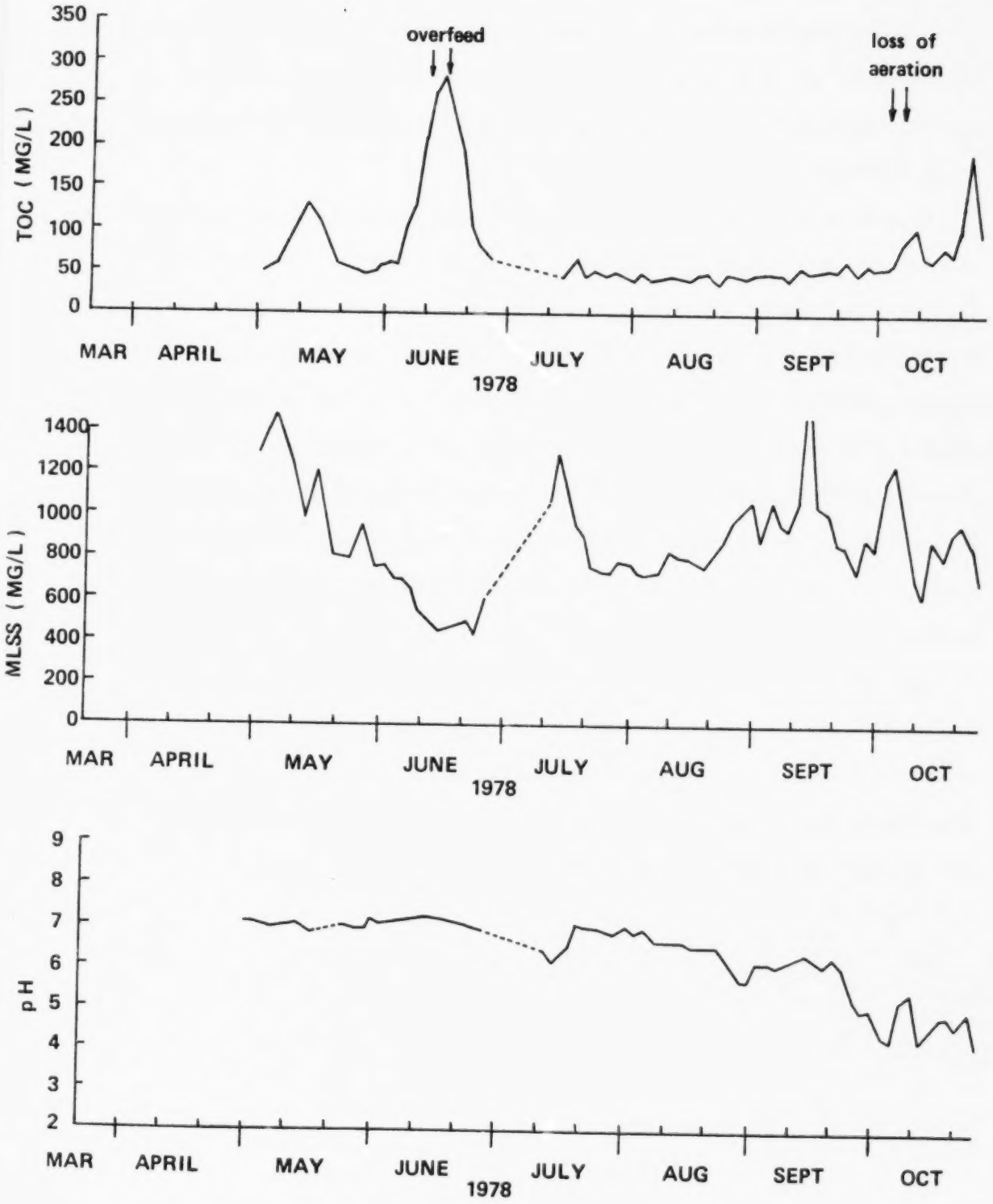


Figure 17. Performance characteristics of the second 20-day reactor.

operational data suggested that they had reached approximate steady-state performance, and intensive data collection for this pattern of operation was initiated in early June. Five sets of filtered samples from the reactors were analyzed for BOD, COD, nitrogen species, and phosphorus as shown in Table 7.

It had been planned that the analyses would be continued at intervals of two days over a period of at least two weeks. If the data then indicated that steady-state had, in fact, been attained, intensive sampling would have been discontinued and the operations modified to another set of reactor conditions. During the intensive sampling period in June, however, the data for TOC and mixed liquor suspended solids indicated clearly that steady-state operation had not been attained. Effluent TOC in all of the reactors rose sharply beginning about June 9. This led to a decision to postpone the intensive analysis program until more consistent performance could be achieved.

The exact cause for the substantial change in performance which occurred in June is unknown. However, a short time earlier, the time clock controlling the feed to the reactors malfunctioned, resulting in an overfeed to the 10- and 20-day reactors. This malfunction was corrected and the feed rate was readjusted for normal operation.

TABLE 7. SUMMARY OF REACTOR PERFORMANCE DURING PERIODS OF INTENSIVE ANALYSIS

Date (1978)	Sample	TOC mg/l	BOD mg/l	COD mg/l	NO_2 mg/l as N	NO_3 mg/l as N	NH_3 mg/l as N	TKN mg/l as N	Total Phosphate mg/l	Ortho-Phosphate mg/l
5/30	Raw Waste		3,520	5,880	0.03	11.0	243	239	423	---
6/5	" "		2,880	5,800						
9/12	" "		4,140	5,450						
5/30	5-day Reactor	430	1,115	1,600	0.005	3.3	228	243	68	46
6/1	"	399	870	1,648			228	273		
6/3	"	463	960	1,728						
6/5	"	469	1,055	1,744						
6/7	"	521	1,100	2,112	0.12	6.8	209	370	42	50
5/30	10-day Reactor	95	179	400	0.064	2.0	234	231	106	99
6/1	"	93	140	360			222	243		
6/3	"	98	171	488						
6/5	"	130	245	532						
6/7	"	143	240	616	0.05	5.6	222	330	35	38
9/8	"	90	43	---						
9/12	"	112	26	275						
9/14	"	112	25	315						
9/16	"	116	33	330						
9/18	"	119	---	320						
5/30	20-day Reactor #1	47	47	340	0.07	5.5	217	242	369	333
6/1	"	64	30	348			231	246		
6/3	"	65	45	352						
6/5	"	70	80	400						
6/7	"	70	52	368	0.07	5.6	225	330	42	41
9/8	"	34	5	---						
9/12	"	51	7	190						
9/14	"	47	8	180						
9/16	"	53	7	210						
9/18	"	57	---	190						
5/30	20-day Reactor #2	57	73	292	0.07	3.2	247	244	435	400
6/1	"	59	18	280			249	254		
6/3	"	57	38	356						
6/5	"	99	170	496						
6/7	"	123	183	552	0.06	4.4	240	290	50	51
9/8	"	39	5	---						
9/12	"	53	5	200						
9/14	"	51	4	195						
9/16	"	54	7	220						
9/18	"	56	---	240						

During July, August, and September, mixed liquor suspended solids and TOC data indicated reasonably steady performance, with the possible exception of the 5-day reactor, which had performed irregularly since startup. In all units there was a pronounced tendency for pH to drift downward during this period, although the change in pH did not appear to affect the stability of the MLSS and effluent TOC. Accordingly, additional samples were taken during September for detailed chemical analysis, as shown in Table 7. Because of its erratic performance, the 5-day reactor was not sampled intensively during this period. The 10- and 20-day reactors produced very low effluent BOD's, indicating that almost all of the biodegradable material had been removed. The COD reductions are consistent with the reduction in TOC exhibited in Figures 14 through 17. The nitrogen and phosphorus measurements indicate that there are sufficient nutrients for biological activity and that microbial growth was not inhibited by a lack of nutrients. The distribution among the nitrogen species shows that no nitrification took place.

Although the performance of the reactors appeared to be reasonably consistent during the September sampling period, the pH was unstable and continued to drift downward as shown in Figures 14 through 17, indicating clearly that steady-state operation had not really been attained. During October, the pH in the reactors reached levels lower than 4.0, causing concern about reactor stability due to the depressed pH. This concern was compounded by sharp rises in effluent TOC following loss of aeration for several hours because of compressor failure. Accordingly, in late October, this series of experiments was terminated.

SUMMARY OF PRELIMINARY RESULTS

Overall performance of the units from March through October may be summarized with a few pertinent observations. All of the reactors showed excellent TOC removals from the feed level of approximately 1,600 mg/l. Figure 18 summarizes TOC removal data for the months of July, August, and September before major excursions in pH were experienced. The 5-day reactor was capable of producing an average effluent TOC of about 200 mg/l, with a range extending from about 80 to 300 mg/l. The 10-day reactor produced an average effluent BOD of about 80 mg/l, with more consistent performance as shown by the narrower range of approximately 60 to 120 mg/l. The two 20-day reactors performed in substantially identical fashion, with effluent TOC's averaging 45 mg/L and a rather narrow operating range of approximately 40-60 mg/l. Table 8 summarizes the average performance of the reactors for the months of July, August, and September taken from the data in Figures 14 through 17 and Table 7.

Due to continued difficulties with pH variations, changes were made in the character of the synthetic wastewater to provide additional buffer capacity and to eliminate acetone in preparing the synthetic feed. The results of this modification are described in the following section.

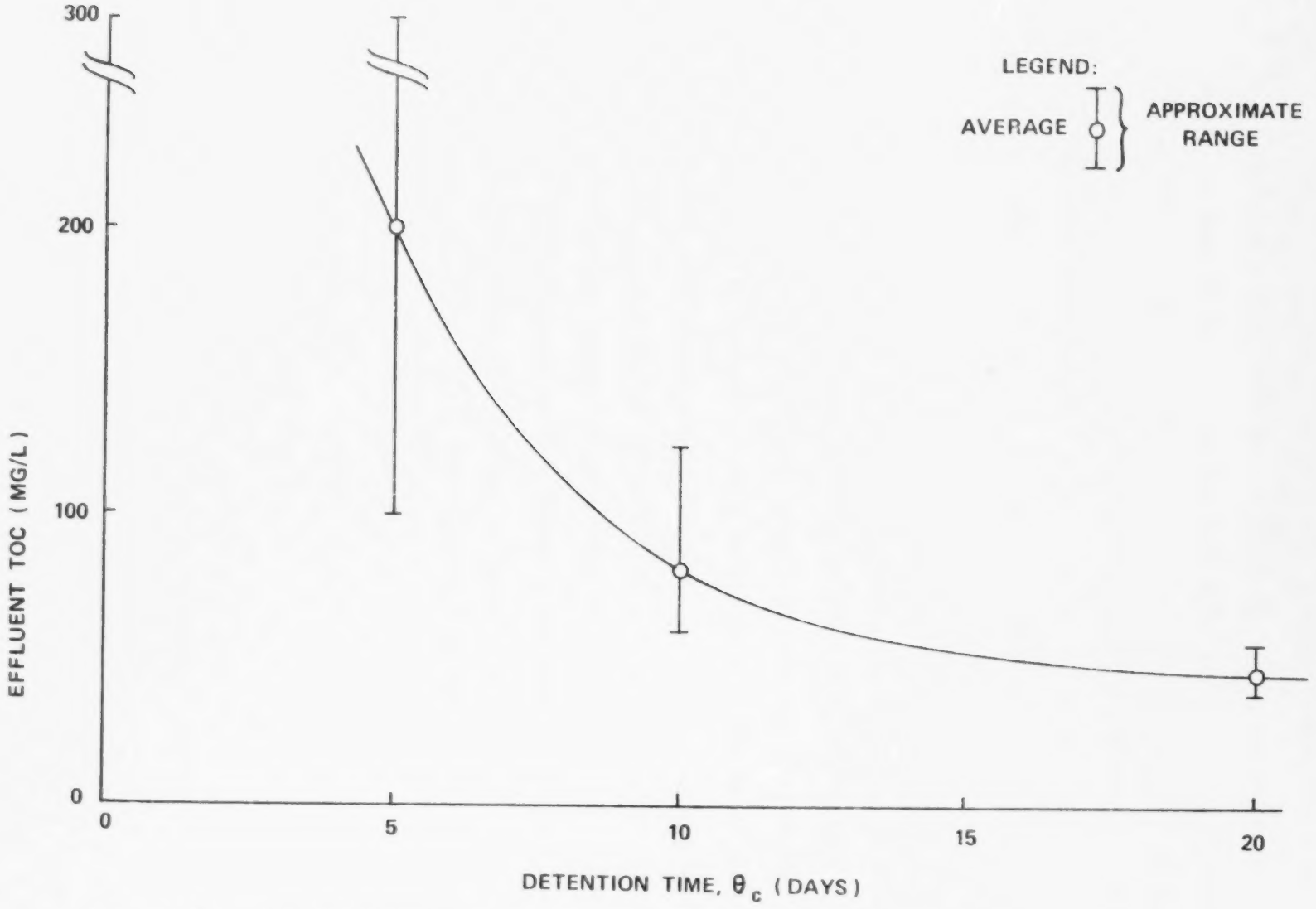


Figure 18. Effect of residence time on reactor performance and stability.

TABLE 8. AVERAGE QUALITY OF EFFLUENT FROM BIOLOGICAL TREATMENT UNITS
(All values in mg/l.)

	Raw Wastewater	Reactor Detention Time (Days)			
		5	10	20	20
BOD	3,510	1,020	32	7	5
COD	5,710	1,770	310	192	214
TOC	1,600	200	80	45	45
MLSS	----	700	900	950	900

SECTION 5

BIOLOGICAL TREATMENT OF SYNTHETIC COAL CONVERSION WASTEWATER:PART 2

OPERATING PROCEDURES

In order to overcome the pH variability and resulting instability of the biological reactors discussed in the previous section, positive pH control was established through the use of a stronger phosphate buffer system. Several other inorganic components were modified as well; a complete listing of the inorganic constituents, including the phosphate buffer, is provided in Table 9. It is unlikely that pH control will be a problem in treating real conversion wastewaters due to the presence of abundant amounts of bicarbonate/carbonate alkalinity in such wastewaters (2).

In addition to providing a stronger buffer system for pH control, the procedure for preparing the organic constituents was modified to eliminate the need for large concentrations of acetone which had been employed in the first phase to solubilize several of the slightly soluble components. Stock solutions of the following form were prepared, depending upon the aqueous solubility of the constituents:

a) organics dissolved in distilled water;

b) organics dissolved in other organics;

c) organics dissolved in methanol.

TABLE 9. CONCENTRATION OF INORGANIC CONSTITUENTS IN QUARTER-STRENGTH SYNTHETIC WASTEWATER

	Concentration, mg/l
NH_4Cl	955
$MgSO_4 \cdot 7H_2O$	5.63
$CaCl_2$	6.88
FeNaEDTA	0.085
$NaHCO_3$	75.0
Phosphate Buffer:	
KH_2PO_4	213
K_2HPO_4	544
$Na_2HPO_4 \cdot 7H_2O$	835

The remaining organic constituents were stored as dry powders and weighed out as needed. Only three constituents were dissolved in methanol, resulting in a substantial reduction in the need for extraneous organic solvents. The TOC attributable to the methanol in the quarter-strength wastewater was approximately 35 to 50 mg/l. The concentrations of the original 28 organic constituents in the synthetic wastewater remained unchanged.

The new organic stock solutions necessitated the make-up of larger feed batches to maintain both wastewater consistency and a reasonable ease of chemical handling. Initially, the feed was prepared in 100-liter batches.

The synthetic wastewater was mixed in a stainless steel tank which was connected to a 5-gallon glass vessel. The feed pumps for the reactors drew wastewater from this glass vessel. Each 100-liter batch lasted for about 10 days. In February 1979 three new reactors were put on line, making a total of seven reactors in operation. In conjunction with the start-up of the new reactors, the volume of the synthetic wastewater batches was increased to 200 liters, which represents the maximum capacity of the system. The 200-liter batches provided continuous feed for approximately 9 days.

The duration of the feed batches led to concerns over possible chemical changes in the character of the wastewater. Chromatogaphic analyses established that some changes do occur but, as reported earlier, these changes appear to be minimal.

During this second period of study, operation of the reactors and the sampling procedures remained essentially unchanged. The only exceptions were in evaluating sludge settleability and in the rates of air flow to the reactors. The sludge volume index (SVI) test (see Section 4) became difficult to conduct because the contents of the reactor became quite dark when the pH was increased to the neutral range. Thus, the solid/liquid interface was difficult to discern. Also, it did not appear that zone settling was occurring. A new operational procedure was substituted for the SVI test to provide an indication of sludge settleability. A one-liter sample of mixed liquor from each reactor was placed in a 1,000 ml graduated cylinder and allowed to settle for 30 minutes. After settling, the top 500 ml was decanted through a glass port constructed in the cylinder. This supernatant liquid was mixed on a magnetic stirrer and a suspended solids

analysis was performed in accordance with the MLSS procedure. A comparison between the MLSS of the supernatant and the MLSS in the reactor provided an operational measure of sludge settleability.

The other modification involved a reduction in the air flow rate to each reactor. Between December 1978 and February 1979, the air flow rate was reduced in several steps from 10 liters per minute to 1.5 or 2 liters per minute, depending upon the reactor. Reactors with hydraulic detention times of 5 days or less received 2 liters of air per minute, while the others received 1.5 liters per minute. The change was undertaken mainly to reduce foaming problems which had developed. The lower air flow rates still provided intimate mixing and a generous supply of dissolved oxygen to meet the metabolic needs of the organisms.

The three new reactors which were started up in February were operated in the same manner as the other four reactors, i.e. completely mixed activated sludge systems with no recycle of biomass. The new reactors were operated at detention times of 3, 7.5, and 40 days. The 7.5- and 40-day reactors were fed intermittently, at 30-minute intervals, while the 3-day reactor was fed continuously. The three new reactors were started up using the effluent from the first four reactors. (Since no settling is provided in the experimental set-up, the effluent contains metabolically-active organisms as well as residual TOC.) The effluent was added to the reactors three times per week over a 10-day period. The reactor contents were aerated until the desired operating volume was developed, after which feeding of the synthetic wastewater commenced at the appropriate rate.

Some mechanical difficulties were encountered at various times during this second study period. These operational malfunctions probably account

for a number of the transient responses in reactor performance. Operational problems are listed in Table 10, along with the reactor(s) affected.

RESULTS

Figures 19 through 24 show the operating characteristics of the 5-, 7.5-, 10-, the two 20-, and the 40-day reactors. The effluent TOC, in general, decreases with increasing retention time, reflecting improved treatment efficiency. (The influent TOC during this period of operation was measured to be 1,040 $\pm$120 mg/l.) It should be noted that the scales for each of the figures are not the same, so that caution must be exercised in comparing the results. No difficulties were encountered in controlling pH due to the increased buffer capacity of the raw feed; the pH held steady at 6.9 to 7.4.

Attempts to treat the wastewater with a 3-day residence time met with failure. Immediately after feeding of the 3-day reactor commenced, the effluent TOC began to rise and within a few days approached the influent TOC. This pattern was observed a second time, implying that the wastewater cannot be treated with such a low sludge age system.

TABLE 10. RECORD OF EQUIPMENT MALFUNCTIONS

DAY	DESCRIPTION	REACTOR(S) AFFECTED
46	loss of air - 24 hrs maximum	all
60	pump malfunction - no feed for 2 days	5-day
62	reactor overfed	5-day
76	loss of air - 12 hrs maximum	all
79	loss of air - 24 hrs maximum	all
80	loss of air - 8 to 10 hrs	all
82	feed off due to flooding	all
105	reactor overfed	20-day
113	reactor underfed	5-day
115	reactor underfed; reactor leak	3-day
119	reactor underfed	3-, 5-, 7.5-day
143	reactor overfed	5-day
146	reactor underfed	5-day
146	loss of air	all
148	reactor overfed	5-day
218	punctured feed line, reactor leak	3-day
220	punctured feed line, reactor leak	40-day

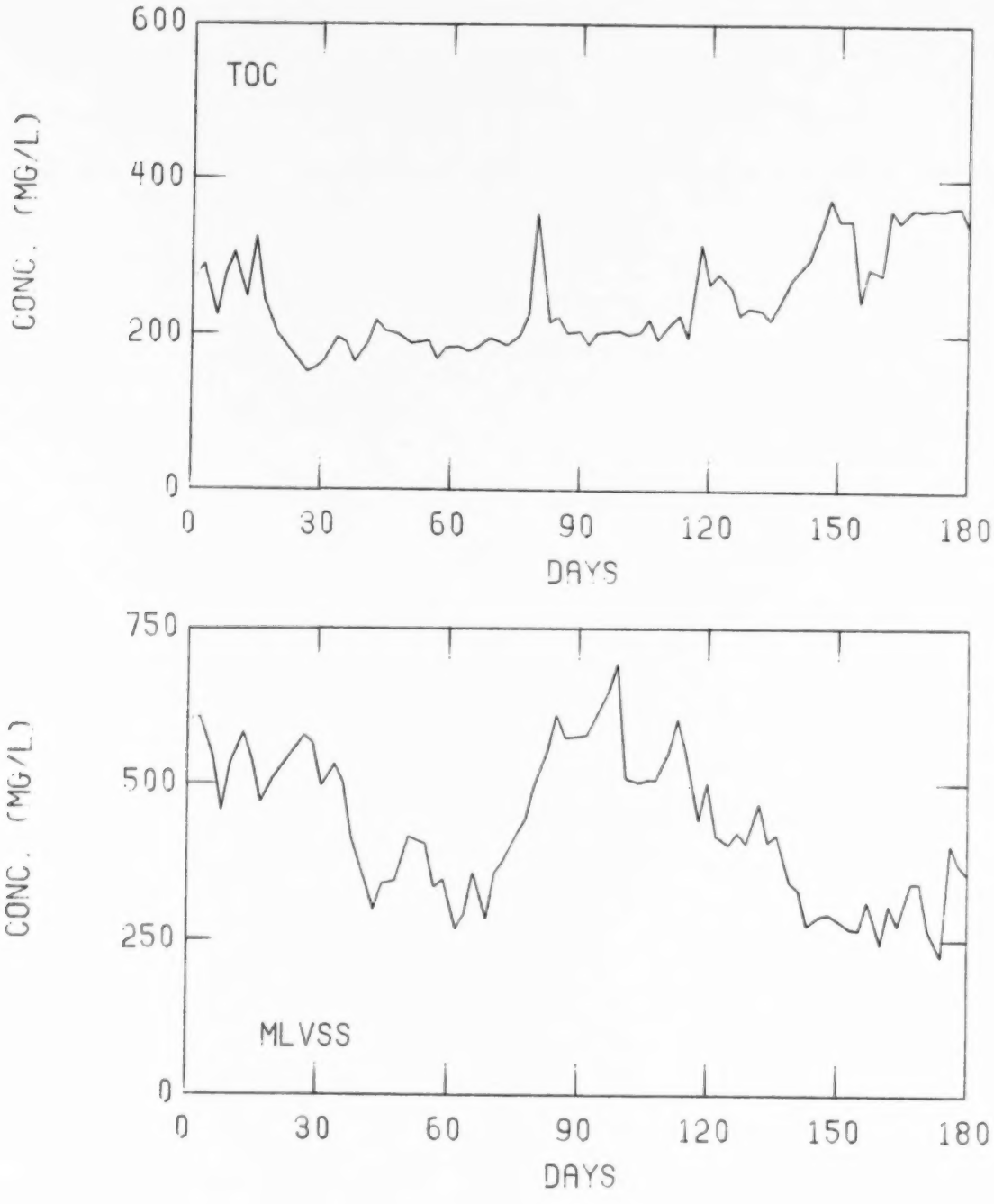


Figure 19. Effluent TOC and mixed liquor volatile suspended solids concentration in 5-day reactor.

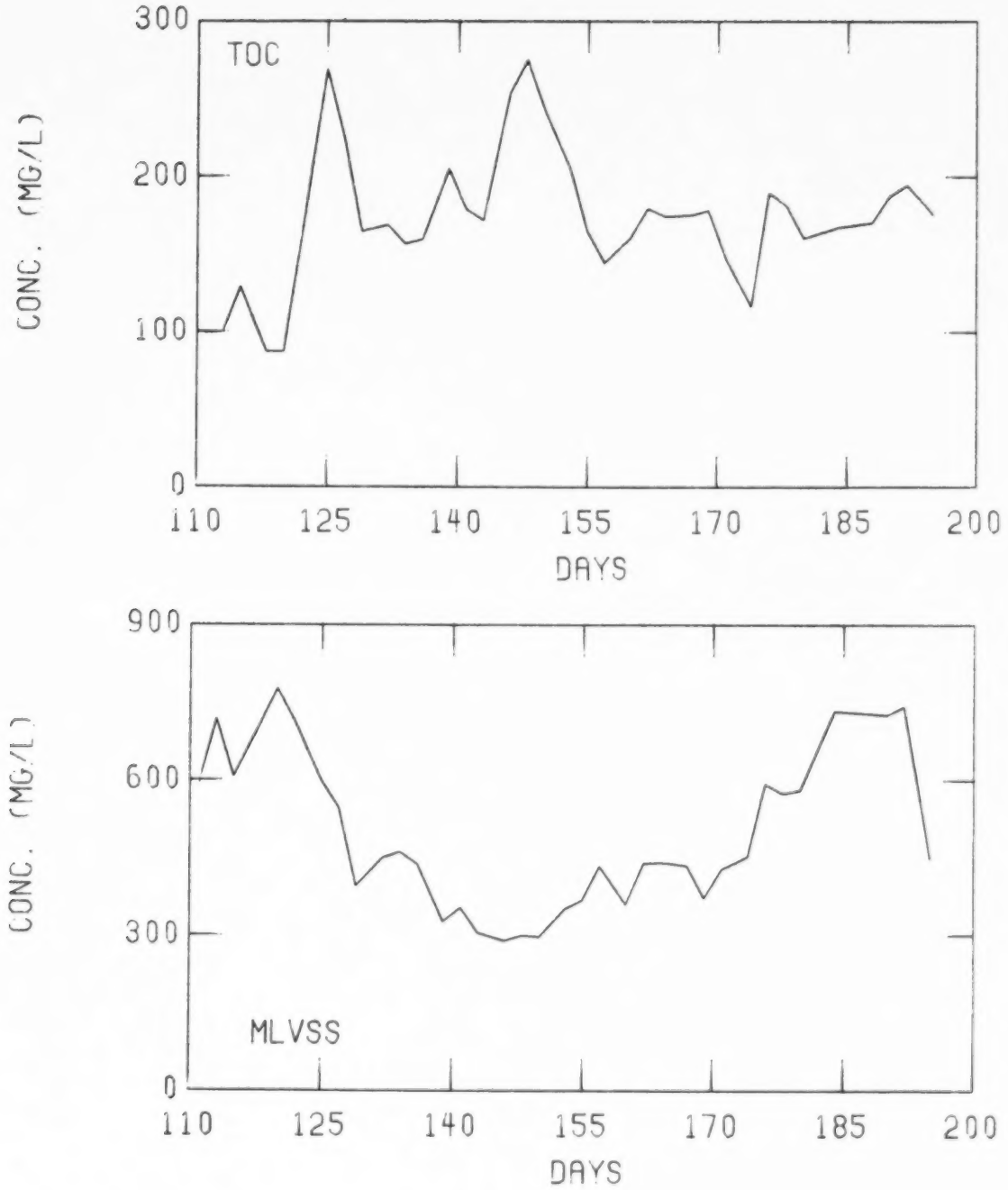


Figure 20. Effluent TOC and mixed liquor volatile suspended solids concentration in 7.5-day reactor.

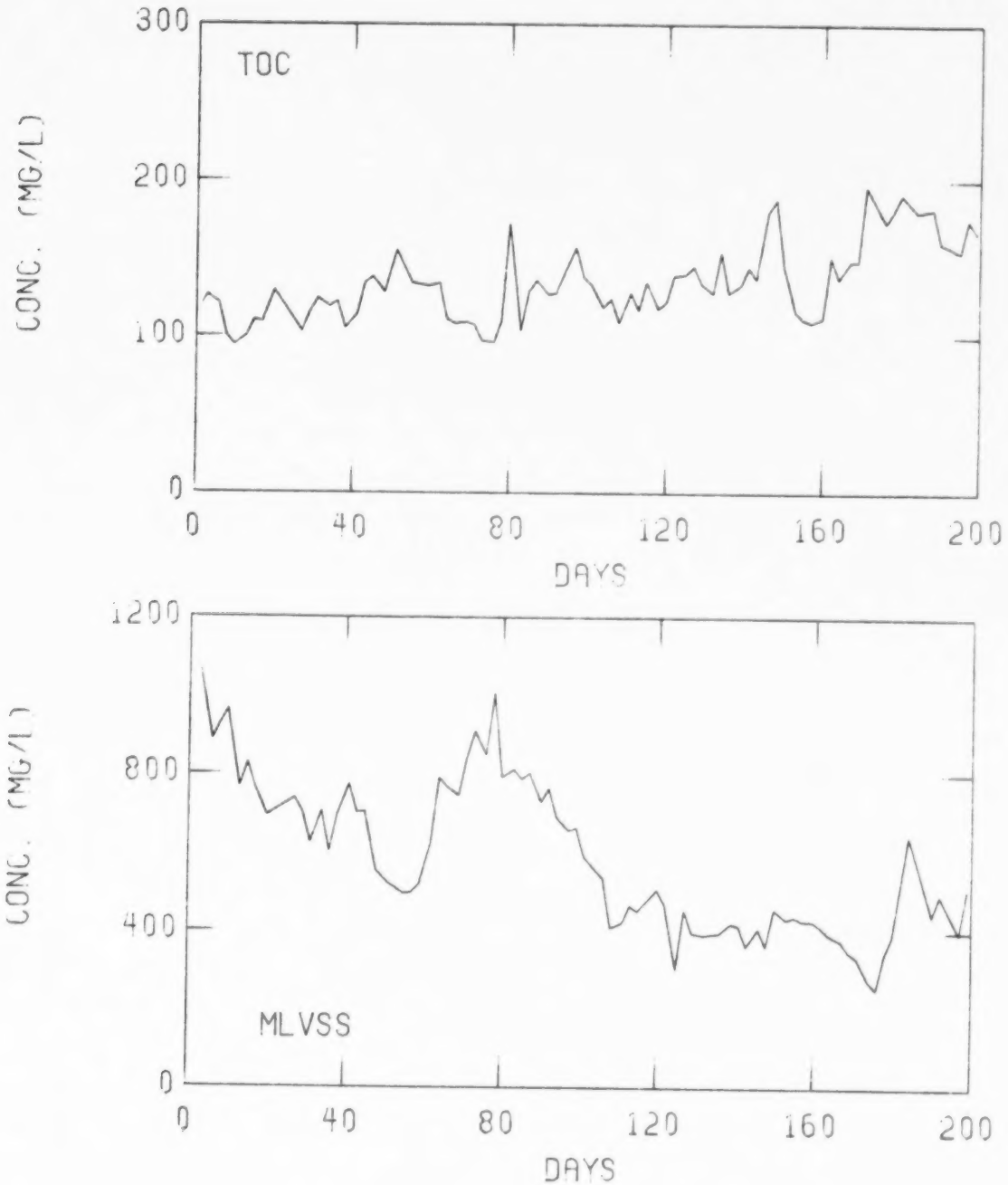


Figure 21. Effluent TOC and mixed liquor volatile suspended solids concentration in 10-day reactor.

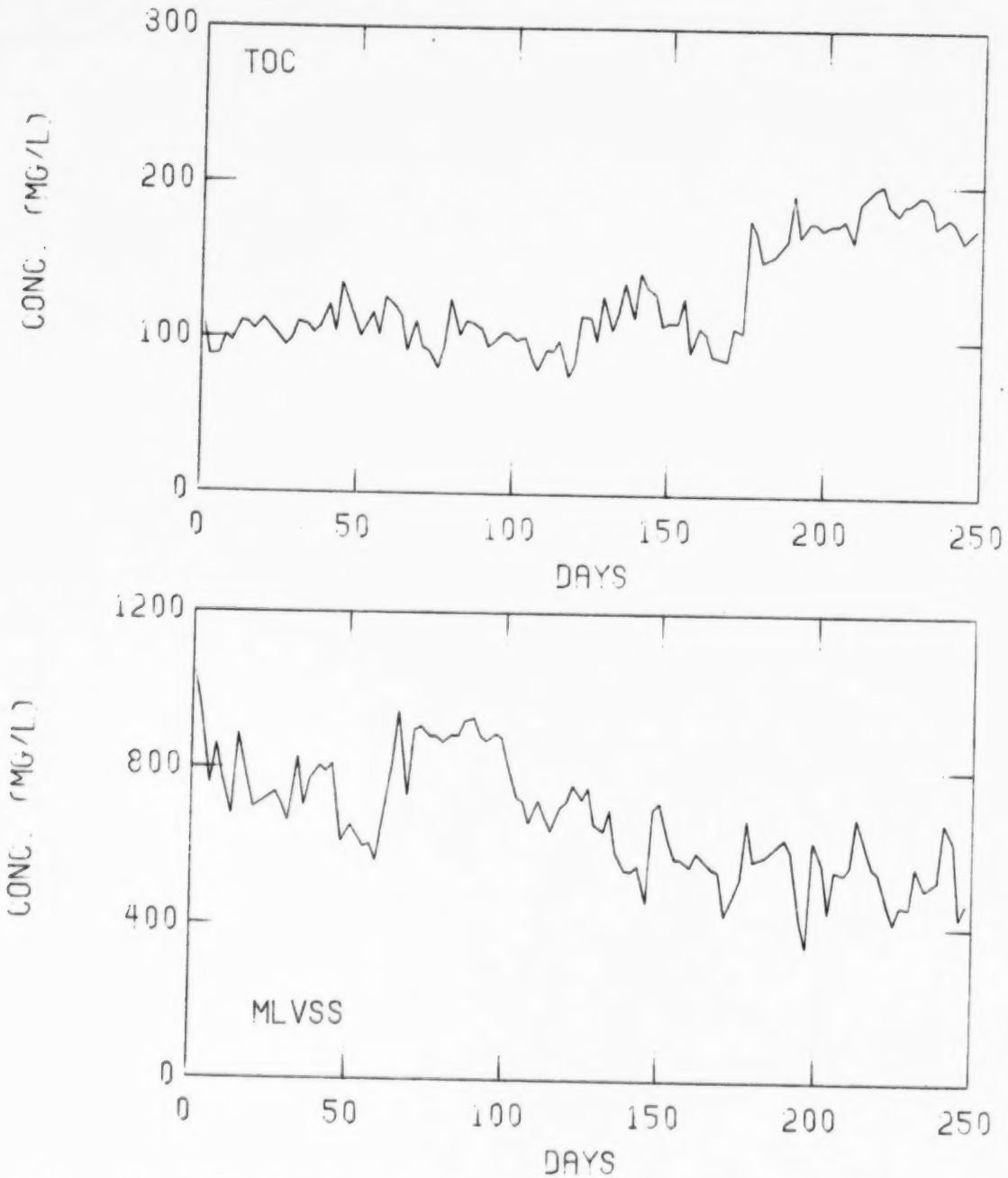


Figure 22. Effluent TOC and mixed liquor volatile suspended solids concentration in first 20-day reactor.

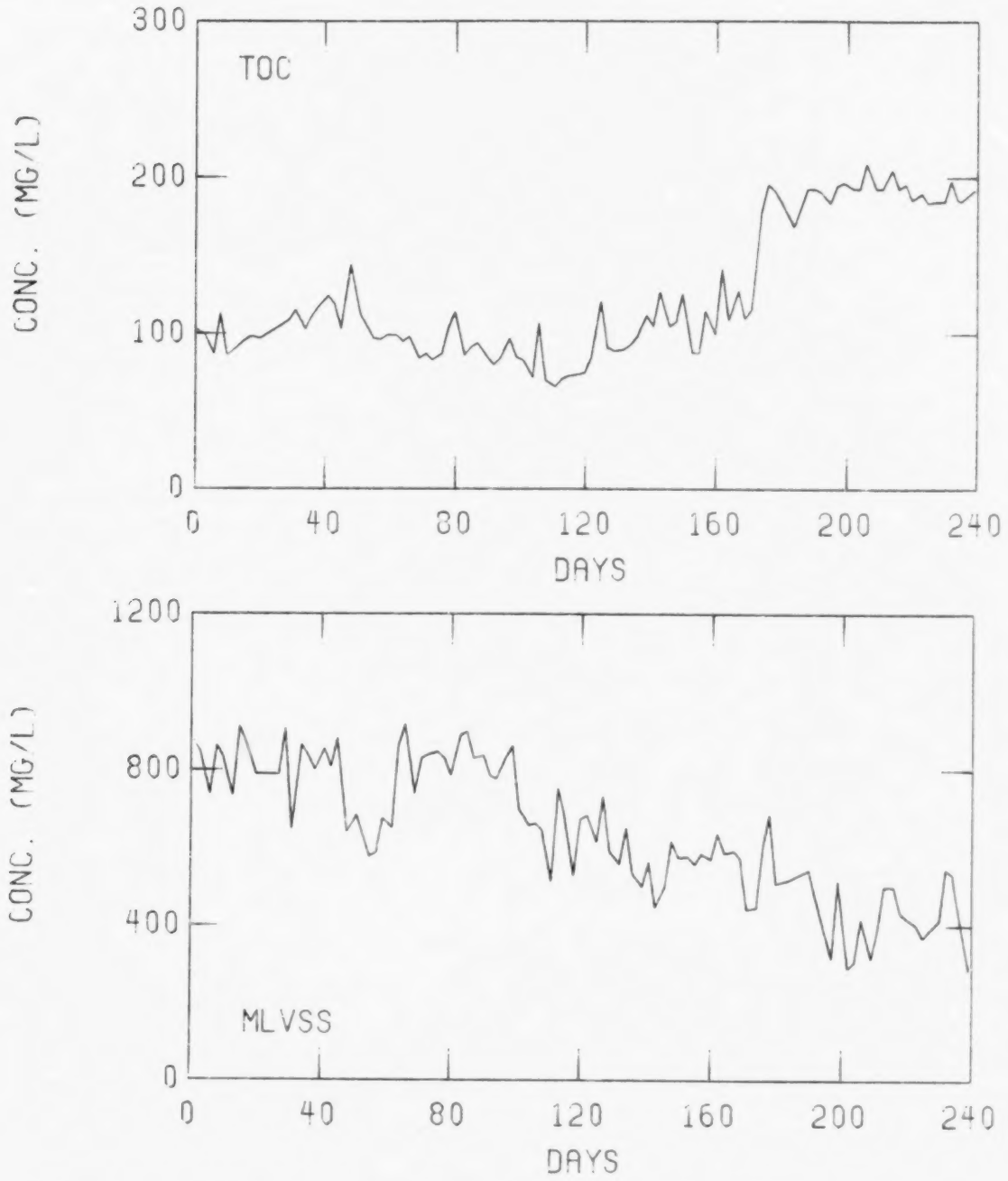


Figure 23. Effluent TOC and mixed liquor volatile suspended solids concentration in second 20-day reactor.

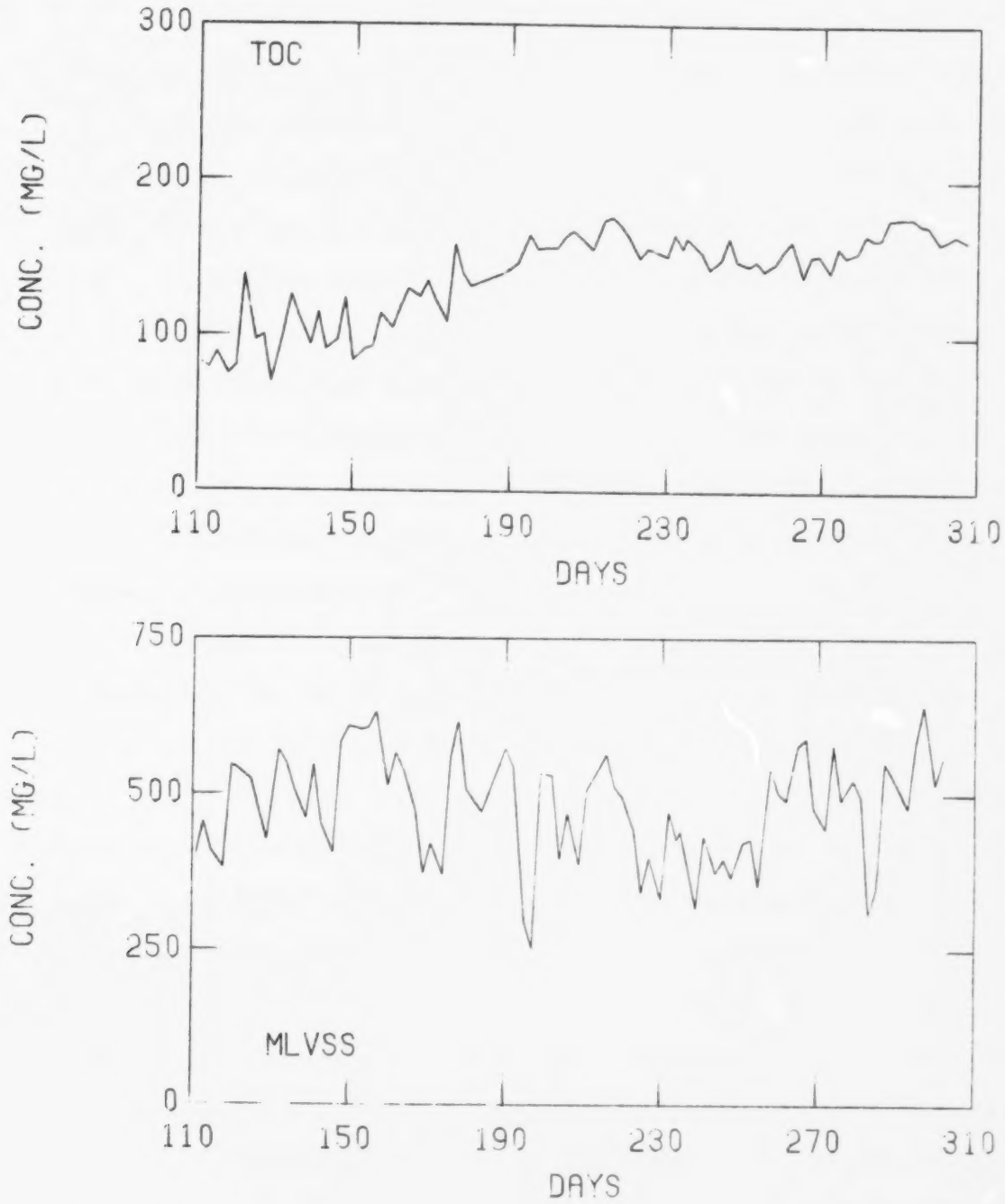


Figure 24. Effluent TOC and mixed liquor volatile suspended solids concentration in 40-day reactor.

A closer look at the TOC data in Figures 19 to 24 shows that, in general, reasonably steady performance was maintained for about 140 to 170 days after which the effluent TOC increased somewhat. In fact, there appears to be a slight upward trend in the TOC data, and a decrease in MLVSS over the period of observation. Accordingly, it may be inappropriate to speak of steady-state behavior, despite the rather consistent performance of the reactors over this observation period of up to 8 months. Some of the observed fluctuations in the TOC and MLVSS data may be attributed to the operational malfunctions as itemized in Table 10. Additionally, significant fluctuations in the ambient temperature began at about the 160th day of operation, marking the change from winter to spring. The external perturbations may have influenced microbial kinetics and altered the biomass population and distribution in the reactors, thereby eliciting the unsteady responses observed in reactor performance. The reason for this shift in performance characteristics is being investigated.

Analyses of the effluent BOD and COD and the concentration of phenols were also made at selected times during this period of investigation and the results are shown in Table 11. These numbers, when compared against the measured influent concentrations of 1,780, 2,830, and 575 mg/l of BOD, COD, and phenols, respectively, reflect the excellent degrees of treatment which were achieved. (Phenols were determined using the 4-aminoantipyrine procedure (8, 9) which responds only to certain of the phenolic constituents.)

Figures 25 to 27 present correlations among effluent BOD, COD, and TOC, illustrating the strong inter-dependence of these 3 parameters for this

TABLE 11. SUMMARY OF REACTOR PERFORMANCE (All values in mg/l.)

A. 5-Day Reactor

DAY	TOC*	BOD	COD	PHENOLS
126	261	112	670	-
131	235	-	-	54
133	232	126	670	-
140	271	235	850	-
147	340	485	1,160	-
154	348	430	1,080	94
161	362	360	825	-
168	363	150	1,025	-
169	361	-	-	33
175	362	186	940	-

B. 7.5-Day Reactor

DAY	TOC*	BOD	COD	PHENOLS
164	175	-	-	0.70
168	176	10	570	--
175	117	3	435	--
185	168	6	445	--
192	195	10	465	--
194	176	-	-	1.16

C. 10-Day Reactor

DAY	TOC*	BOD	COD	PHENOLS
126	140	5	480	-
133	128	5	430	-
140	133	5	460	-
147	180+	57+	700+	71+
154	116	8	460	-
161	112	9	470	0.62
168	148	6	410	-
175	182	6	460	-
185	180	8	380	-
192	158	6	465	-
198	175	11	400	3.3

(continued)

TABLE 11 (continued)

D. 20-Day Reactor

DAY	TOC*	BOD	COD	PHENOLS
126	114	3	310	-
133	106	2	370	0.43
136	136	-	-	0.35
140	114	4	355	-
147	129	2	320	-
150	111	-	-	0.35
154	111	2	360	-
157	92	-	-	0.29
161	108	3	350	-
168	88	2	400	-
175	105	3	420	-
185	155	2	415	-
192	167	1	385	-
196	176	-	-	0.19
198	176	3	420	-
203	175	-	-	-
204	175	-	-	0.18
210	164	3	450	-
217	199	-	-	-
218	201	-	-	0.22
224	182	-	460	-
226	188	3	-	-
231	194	4	465	-
233	193	-	-	0.25

E. 40-Day Reactor

DAY	TOC*	BOD	COD	PHENOLS
193	147	-	340	-
198	156	1	345	-
205	164		-	0.11
210	161	2	420	-
212	156	-	-	0.18
219	172	-	-	0.12
224	151	-	430	-
226	157	1	-	0.15
231	152	2	400	-
240	154	-	-	0.10
252	146	1	375	-
254	149	-	-	0.11
259	148	1	-	-
273	142	3	-	-
282	166	-	-	0.09

+Data questionable; all values are high for this date, reflecting probable equipment malfunction.

*Many of the TOC measurements reported in the Table were made on samples taken the day before or the day after the date indicated.

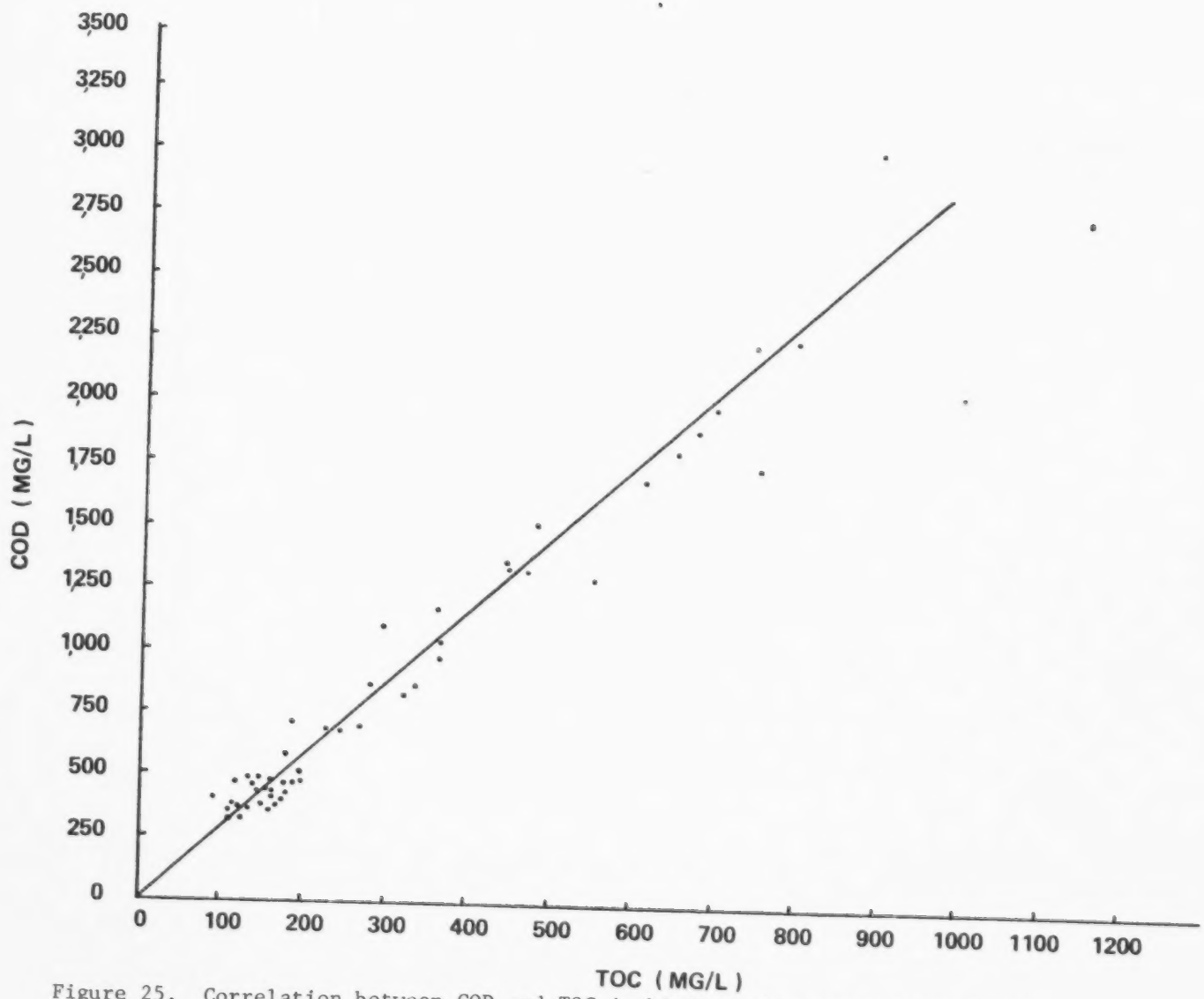


Figure 25. Correlation between COD and TOC in biologically-treated wastewater.

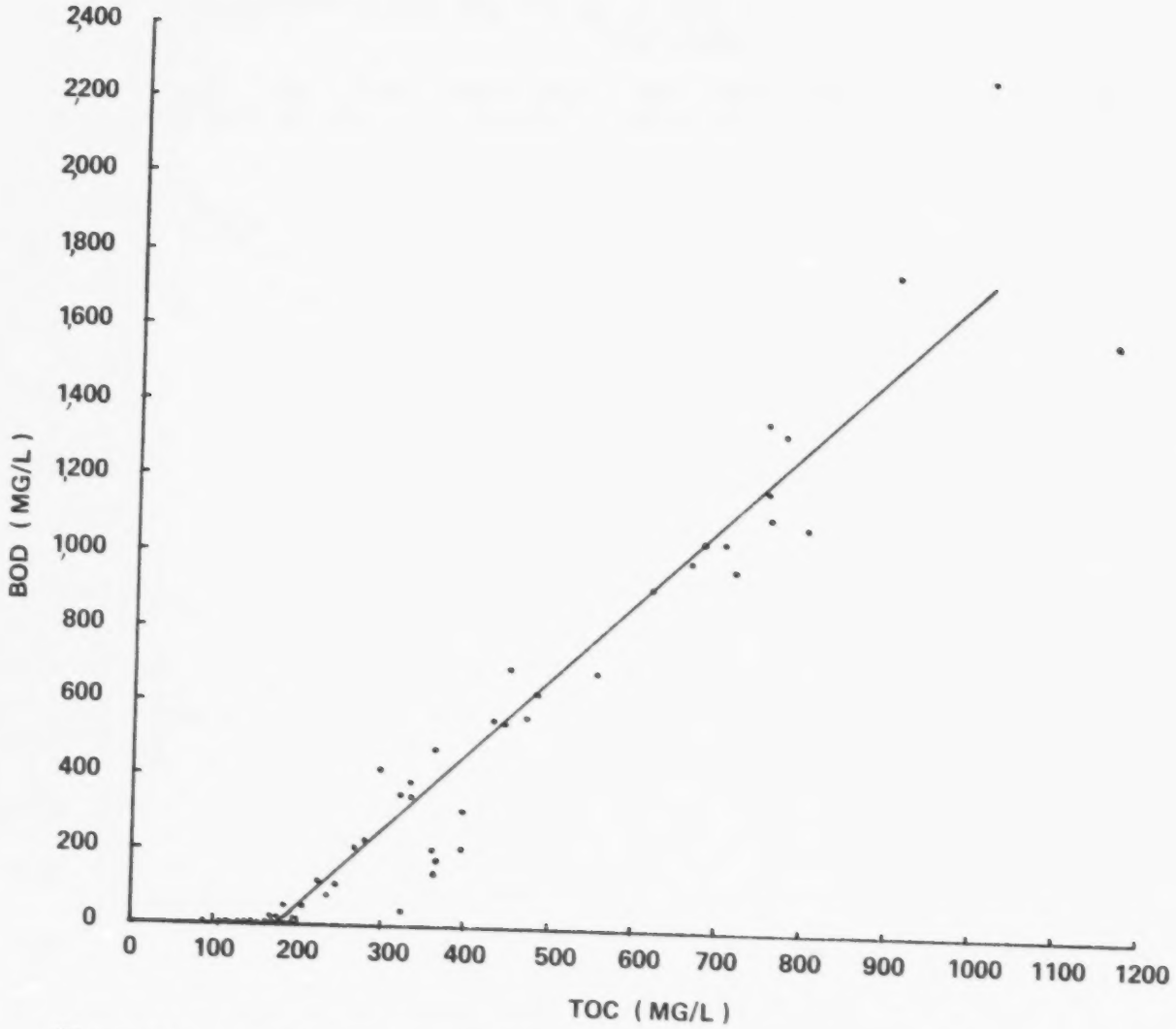


Figure 26. Correlation between BOD and TOC in biologically-treated wastewater.

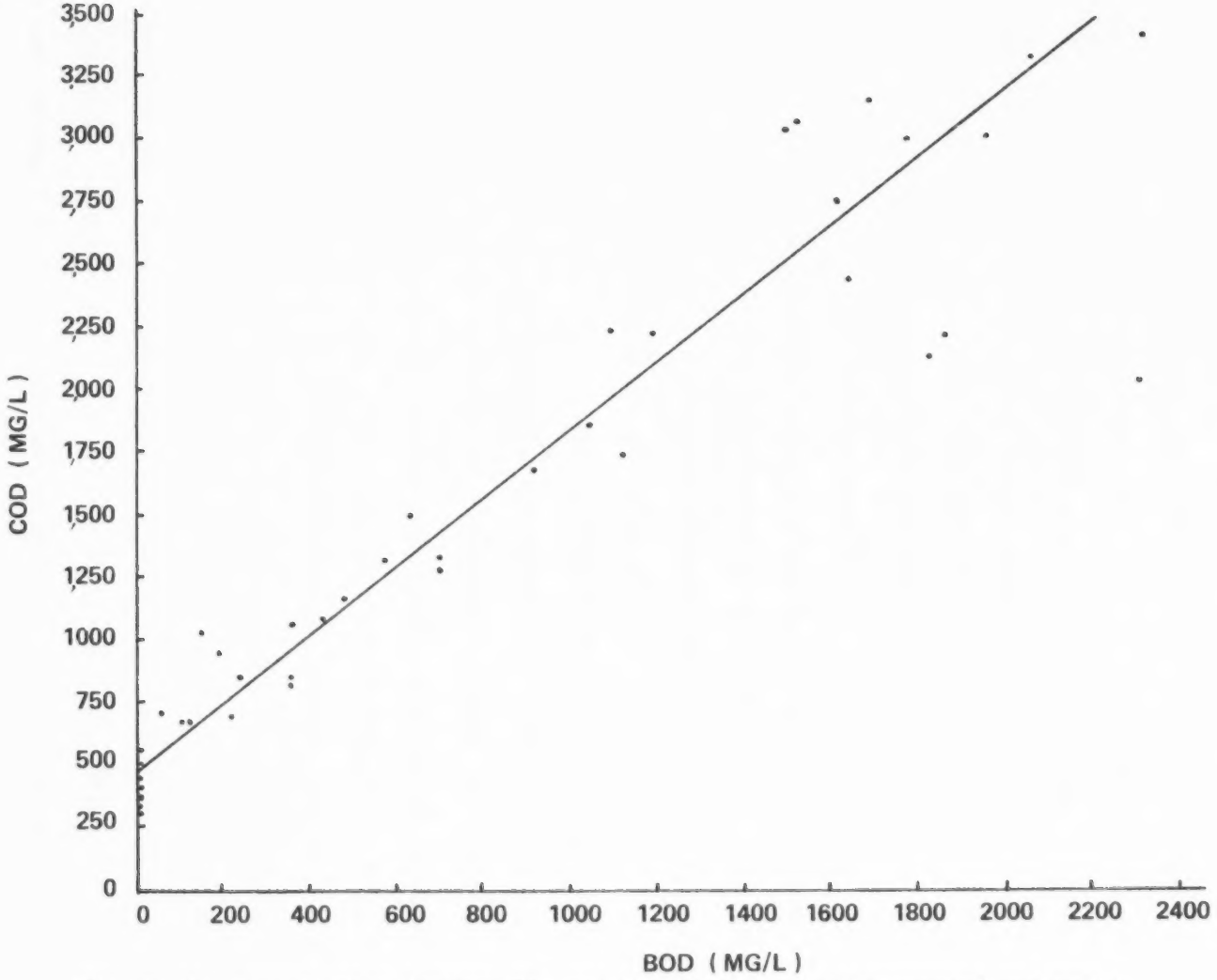


Figure 27. Correlation between BOD and COD in biologically-treated wastewater.

wastewater. The following relationships were developed from these plots:

$$TOC = 190 + 0.47\ (BOD)$$

$$COD = 2.57\ (TOC) + 60$$

$$COD = 642 + 1.16\ (BOD)$$

The cluster of data in the low BOD range (<25 mg/l) were omitted in developing these correlations. Consequently, the linear correlations cannot be applied to effluent BOD concentrations less than 25 mg/l.

In attempting to summarize reactor performance over this observation period, it was necessary to divide some of the data into separate periods during which reasonably steady-state performance was observed. The TOC data shown in Figures 19 to 24 were used for this purpose. For example, Figure 19 shows consistent performance of the 5-day reactor from the 20th day to the 115th day, with an average TOC of 199 $\pm$30 mg/l. After the 139th day, the average TOC was reasonably constant at 332 $\pm$40 mg/l. As indicated above, the reason for this shift is not clear at this time but is believed to be related to conditions external to the reactors, such as temperature changes. Accordingly, Table 12 lists the periods of apparent steady-state operation, and Table 13 summarizes the effluent data during these steady-state periods. The summary results are plotted in Figures 28 to 31. It is clear that the removals of TOC, COD, BOD and phenols improve with increasing sludge age or residence time in the reactors. BOD and phenols are completely removed after 10 to 15 days, while the extent of TOC and COD removal is essentially complete after the same time period, leaving residual non-biodegradable TOC and COD concentrations of approximately 100 to 160 and 350 to 400 mg/l, respectively. These residuals cannot be compared directly with the correlations above, since the correlations were

TABLE 12. PERIODS OF STEADY STATE PERFORMANCE

REACTOR	PERIOD 1	PERIOD 2
5-day	day 20 - day 115	day 139 - day 180
7.5-day	day 111 - day 195	
10-day	day 1 - day 143	day 146 - day 199
20-day, #1	day 1 - day 174	day 176 - day 248
20-day, #2	day 1 - day 171	day 174 - day 239
40-day	day 111 - day 174	day 176 - day 307

TABLE 13. SUMMARY OF AVERAGE STEADY STATE REACTOR PERFORMANCE
(all values in mg/l)

First Steady State Period

REACTOR	MLVSS	TOC	BOD	COD	PHENOLS
5-day	470	199	---	---	------
7.5-day	495	173	7.3	480	0.5
10-day	650	125	5	455	------
20-day, #1	725	106	2.6	350	------
20-day, #2	710	99	---	---	------
40-day	500	104	---	---	------

Second Steady State Period

REACTOR	MLVSS	TOC	BOD	COD	PHENOLS
5-day	305	332	310	980	65
10-day	410	158	7.7	435	2.0
20-day #1	535	179	2.7	430	0.30
20-day #2	445	191	---	---	---
40-day	470	157	1.6	386	0.12

Raw Feed Characteristics

	MLVSS	TOC	BOD	COD	PHENOLS
Feed	-----	1,040	1,780	2,830	575

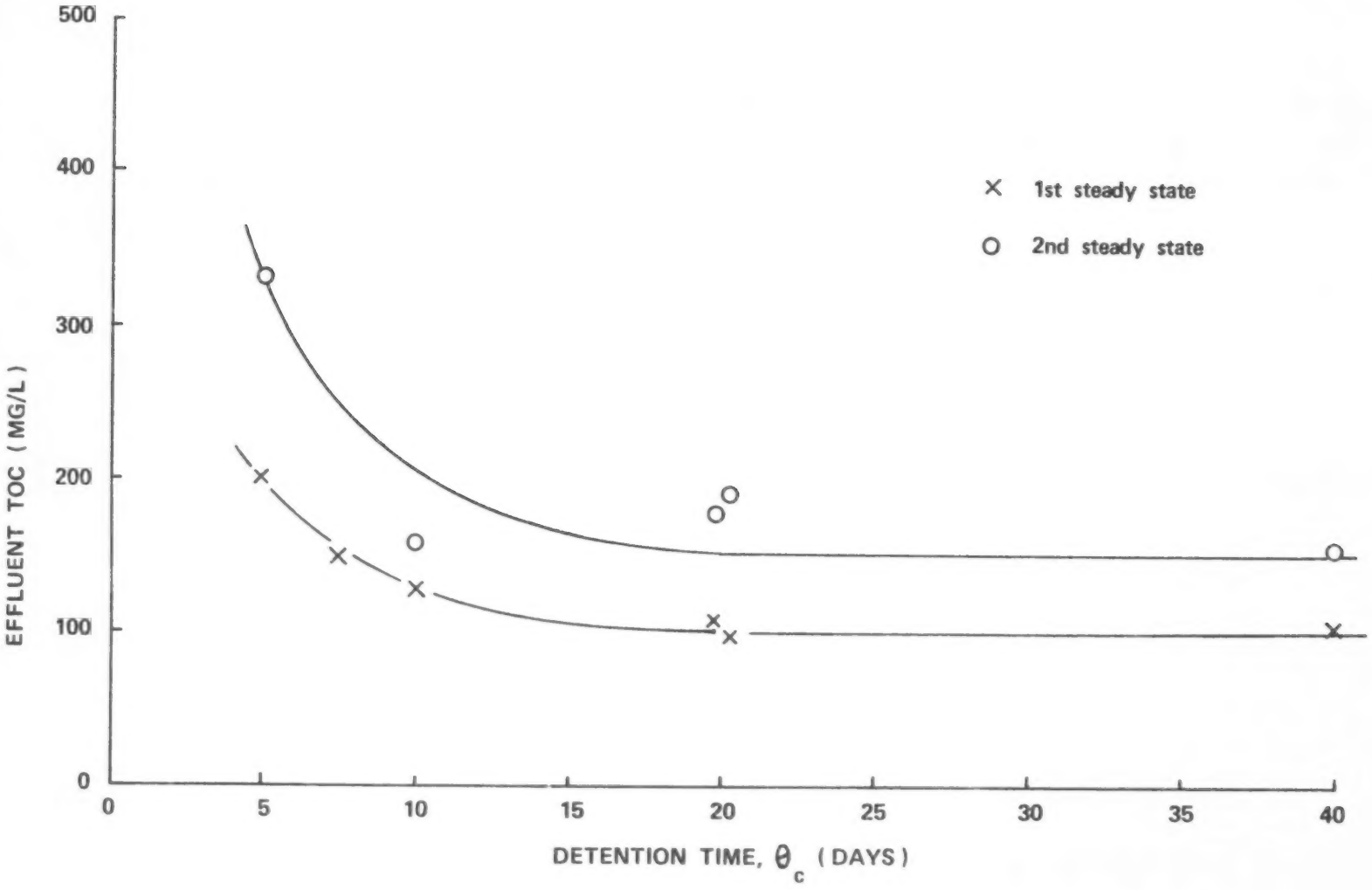


Figure 28. Effect of residence time on TOC removal.

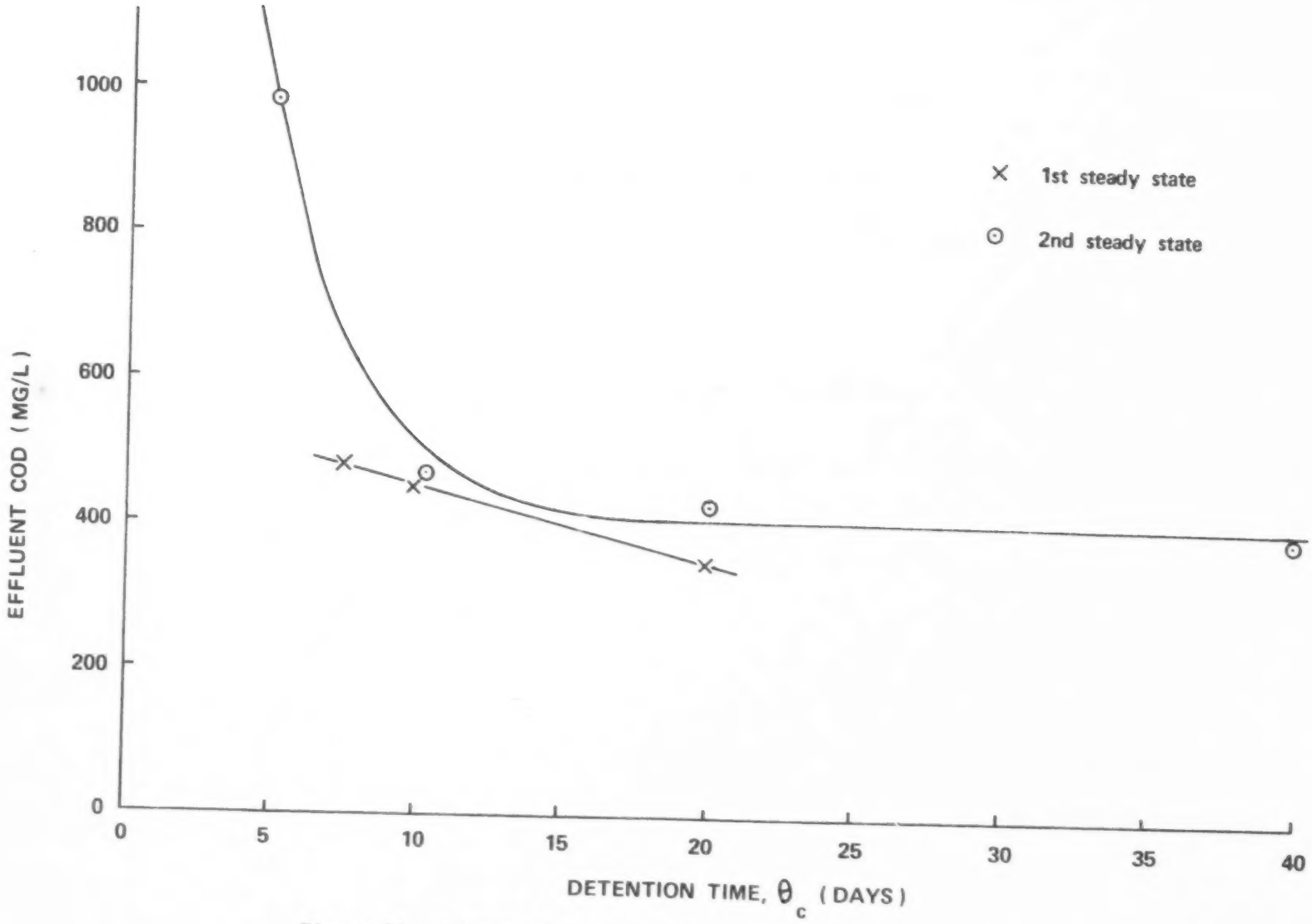


Figure 29. Effect of residence time on COD removal.

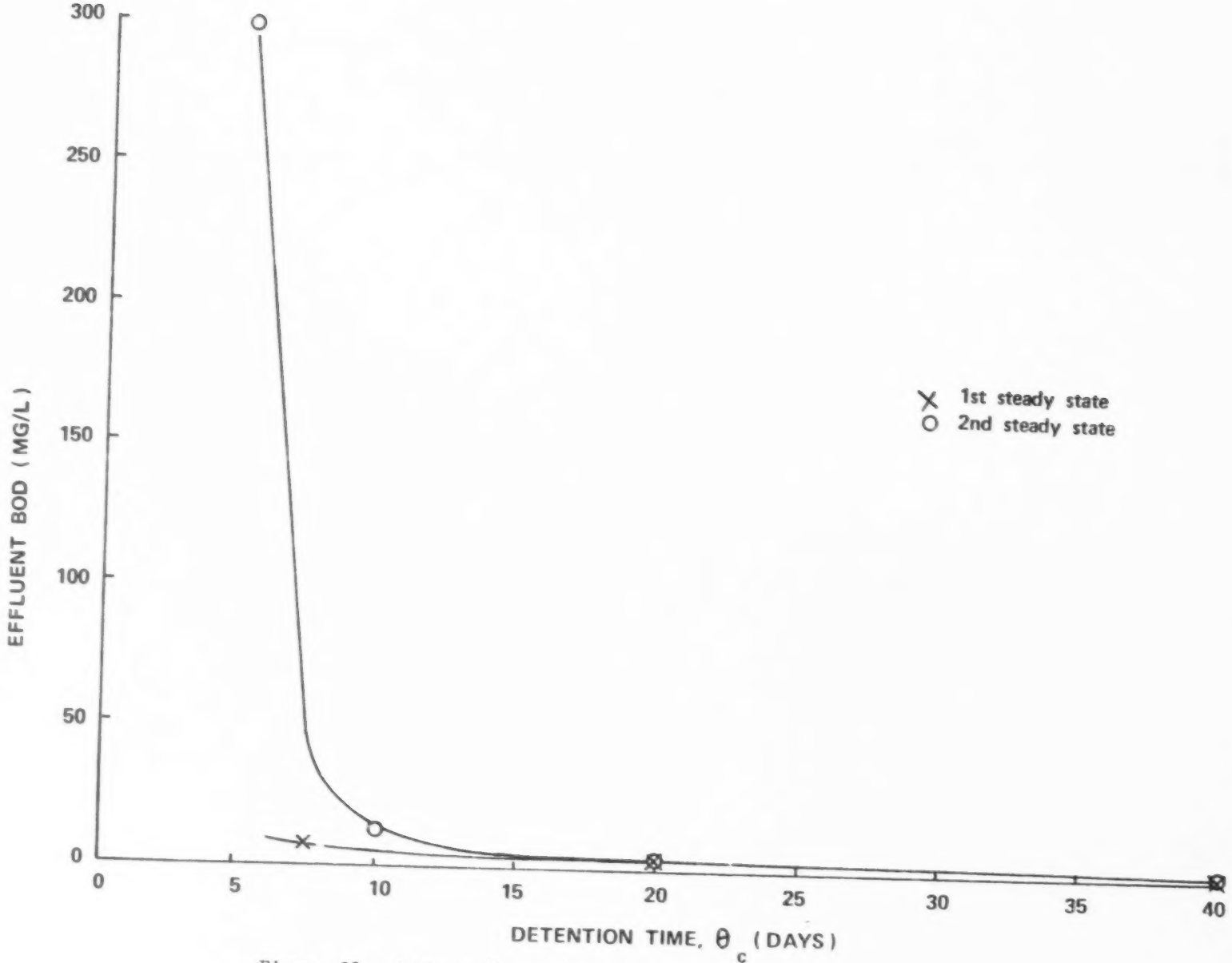


Figure 30. Effect of residence time on BOD removal.

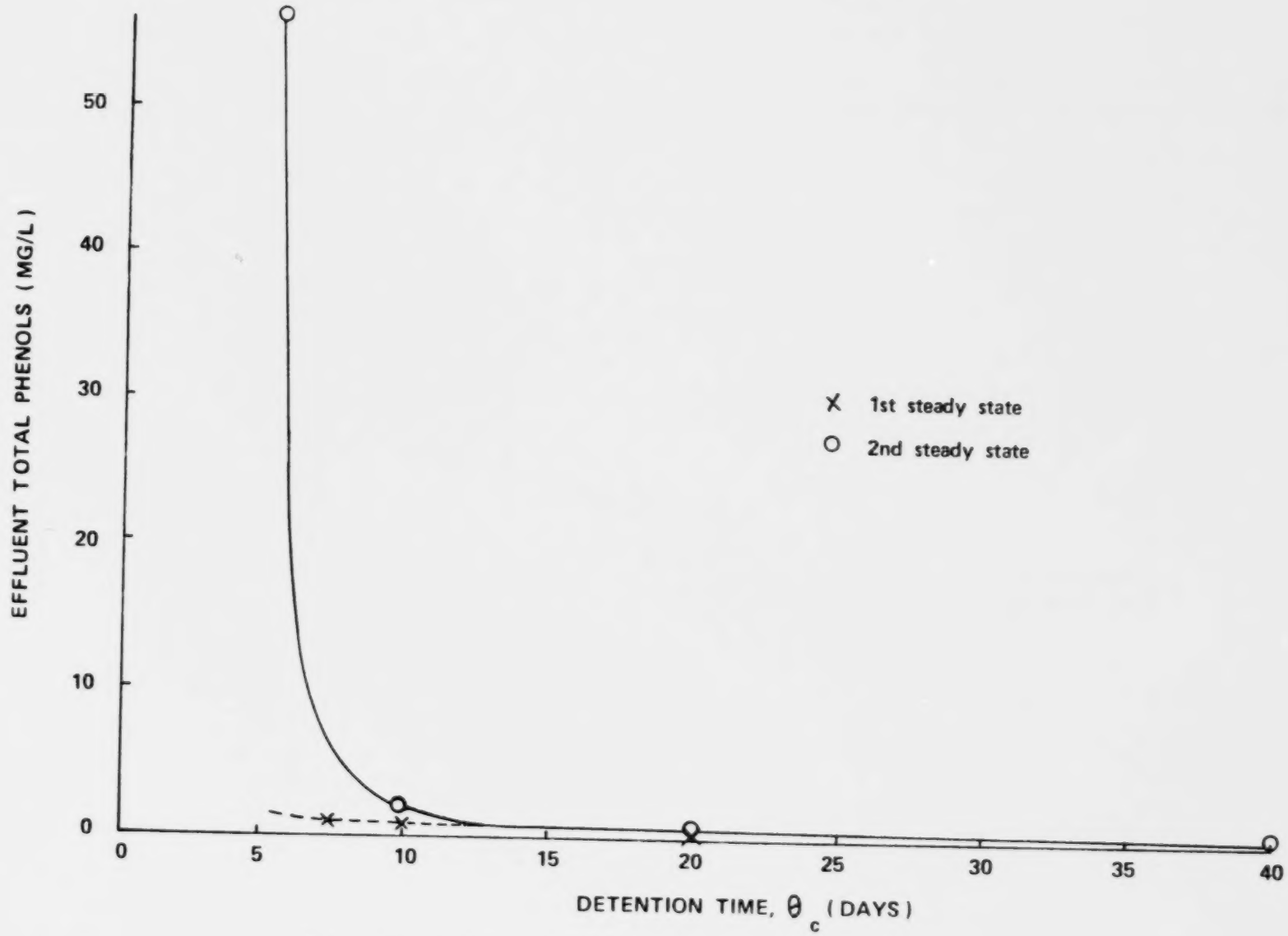


Figure 31. Effect of residence time on phenols removal.

not developed for low effluent BOD concentrations. (See Section 7 for further discussion of these residuals.)

Comparison of the residual TOC and COD for this second period of operation with the residuals discussed in Section 4 (e.g. Figure 18) for the first period of reactor operation show a better degree of treatment (lower TOC residuals) for the first period. It is believed, in view of the results reported in Section 3, that some of the organics from the first period of study were precipitated at the low pH at which the reactors were operated (approximately pH 4). Hence, removal of organics during the first period of study may not have been entirely through a biological degradation mechanism.

For reasons which cannot yet be explained, after 6 to 8 months of relatively consistent performance, all of the reactors with the exception of the 40-day reactor began to fail. The effluent TOC, COD, BOD, and phenols began to increase, approaching the influent concentrations, and oxygen-uptake measurements showed a significant reduction in metabolic activity. The cause of this sudden failure is not known at present, but in view of the long period of relatively consistent behavior, it is believed that the failure is attributable to problems in the mode of operation of the reactors and not to any fundamental problems in the biochemistry of the system, i.e. it is believed that the synthetic wastewater is biologically treatable. This point is being investigated further and will be addressed in future reports.

SECTION 6

KINETIC ANALYSIS OF BIOLOGICAL TREATABILITY DATA

In order to design an activated sludge process for treatment of coal conversion wastewater, the parameters describing the kinetics of microbial growth and substrate utilization for the given wastewater must be determined. The data collected to date can be used to make a preliminary determination of these requisite microbial growth coefficients as follows:

The kinetics of microbial growth can be described by the equation (11)

$$dX/dt = y\ dS/dt - k_d X \qquad (1)$$

where

X = concentration of microorganisms (biomass), in mg of mixed liquor volatile suspended solids (MLVSS) per liter;

S = substrate concentration, in mg/l, on a BOD, COD, or TOC basis;

t = time, in days;

y = microbial yield coefficient, in mg of biomass (MLVSS) produced per mg of substrate (on a BOD, COD, or TOC basis) consumed;

k_d = microbial die-away coefficient, in days $^{-1}$.

Taking finite differences in Equation 1 and dividing through by $\bar{X}$, the mean biomass concentration over the time period Δt, one gets

$$(\Delta X/\Delta t) / \bar{X} = y\ (\Delta S/\Delta t) / \bar{X} - k_d \qquad (2)$$

For the continuous-flow, completely-mixed reactors used in this investigation, $\bar{X}$ is the steady-state biomass concentration in each reactor, and Δt is the detention time of the reactor. Equation 2 can be re-written

as

$$1/\Theta_c = y\ U - k_d \qquad (3)$$

Here, Θ_c can be defined as the mean cell residence time, solids retention time, or sludge age, and is equal to the steady-state quantity of biomass in the reactor, divided by the rate of biomass production. Θ_c has units of time, and for reactor operation with no recycle of biomass, the mean cell residence time is equal to the hydraulic retention time. The quantity U in Equation 3 is defined as the process loading factor, or food to microorganism ratio, and is equal to the quantity of substrate consumed (ΔS) during the given reactor detention period (Δt) divided by the steady-state biomass concentration (compare Equations 2 and 3). The process loading factor can be computed on a BOD, COD, or TOC basis. Hence, if the reciprocal of the sludge age is plotted against the process loading factor in accordance with Equation 3, a straight line should result and the microbial kinetic coefficients y and k_d can be determined.

The kinetics of substrate utilization can be described by the equation (11)

$$dS/dt = (kSX)/(K_s + S) \qquad (4)$$

where

k = specific substrate utilization rate, in mg of substrate (on a BOD, COD, or TOC basis) per mg of biomass (MLVSS) per day;

K_s = Michaelis-Menten coefficient or half-velocity constant, in mg/l, on a BOD, COD, or TOC basis.

TABLE 14. CALCULATED PROCESS LOADING FACTORS FOR BIOLOGICAL REACTORS

A. FIRST PHASE OF REACTOR OPERATION

Process Loading Factor	Θ_c, DAYS			
	5	10	20	20
U_B, mg BOD/mg MLVSS-day	0.71	0.39	0.18	0.19
U_C, mg COD/mg MLVSS-day	1.13	0.60	0.29	0.31
U_T, mg TOC/mg MLVSS-day	0.40	0.17	0.082	0.080

B. SECOND PHASE OF REACTOR OPERATION

i) First steady-state period

Process Loading Factor	Θ_c, DAYS					
	5	7.5	10	20	20	40
U_B, mg BOD/mg MLVSS-day	----	0.48	0.27	0.12	--	--
U_C, mg COD/mg MLVSS-day	----	0.63	0.36	0.17	--	--
U_T, mg TOC/mg MLVSS-day	0.36	0.23	0.14	0.064	0.066	0.047

ii) Second steady-state period

Process Loading Factor	Θ_c, DAYS				
	5	10	20	20	40
U_B, mg BOD/mg MLVSS-day	1.17	0.43	0.17	-----	0.094
U_C, mg COD/mg MLVSS-day	1.22	0.58	0.23	-----	0.13
U_T, mg TOC/mg MLVSS-day	0.47	0.22	0.081	0.096	0.047

The other terms are as defined for Equation 1. Again, taking finite differences and dividing through by $\bar{X}$, the mean biomass concentration over the time period Δt, one gets

$$(\Delta S/\Delta t)/\bar{X} = U = (kS)/(K_s + S) \qquad (5)$$

At low substrate concentrations, where $S << K_s$, a plot of U, the process loading factor or food to microorganism ratio, vs S should be linear, with

a slope of k/K_s. Further, Equation 5 can be manipulated to yield

$$1/U = [(K_s/k) \cdot 1/S] + 1/k \qquad (6)$$

Hence, if the reciprocal of the process loading factor is plotted against the reciprocal of the steady-state effluent concentration, a straight line should result and the kinetic coefficients k and K_s can be determined.

Using the "steady state" data summarized in Tables 8 and 13 for the two series of biological treatability studies described in Sections 4 and 5, the process loading factors for each of the reactors were calculated and are listed in Table 14. The linear plots of the combined data from all phases of operation, in accordance with Equation 3, are shown in Figures 32 to 34. The yield coefficients computed from the slopes of the straight lines are 0.45, 0.18, and 0.16 based upon TOC, BOD, and COD utilization, respectively. The die-away coefficient, determined from the intercept at zero-loading, is a negative number (-0.015 to -0.03 $days^{-1}$) which casts some doubt as to the accuracy of the "steady state" values or the validity of the model.

Figures 35 to 37 are plots of the process loading factor vs residual substrate concentration in accordance with Equation 5 for the data from the second-phase of reactor operation. The plots appear to be linear at the lower concentrations, as would be the case if $S << K_s$. If the non-biodegradable portion of the TOC and COD, determined from the x-intercepts in Figures 36 and 37, are subtracted from the TOC and COD effluent values, and the reduced data plotted in accordance with Equation 6, the linear relationships illustrated in Figures 38 to 40 result. Alternative linear modifications of Equation 5 have also been tested, for example:

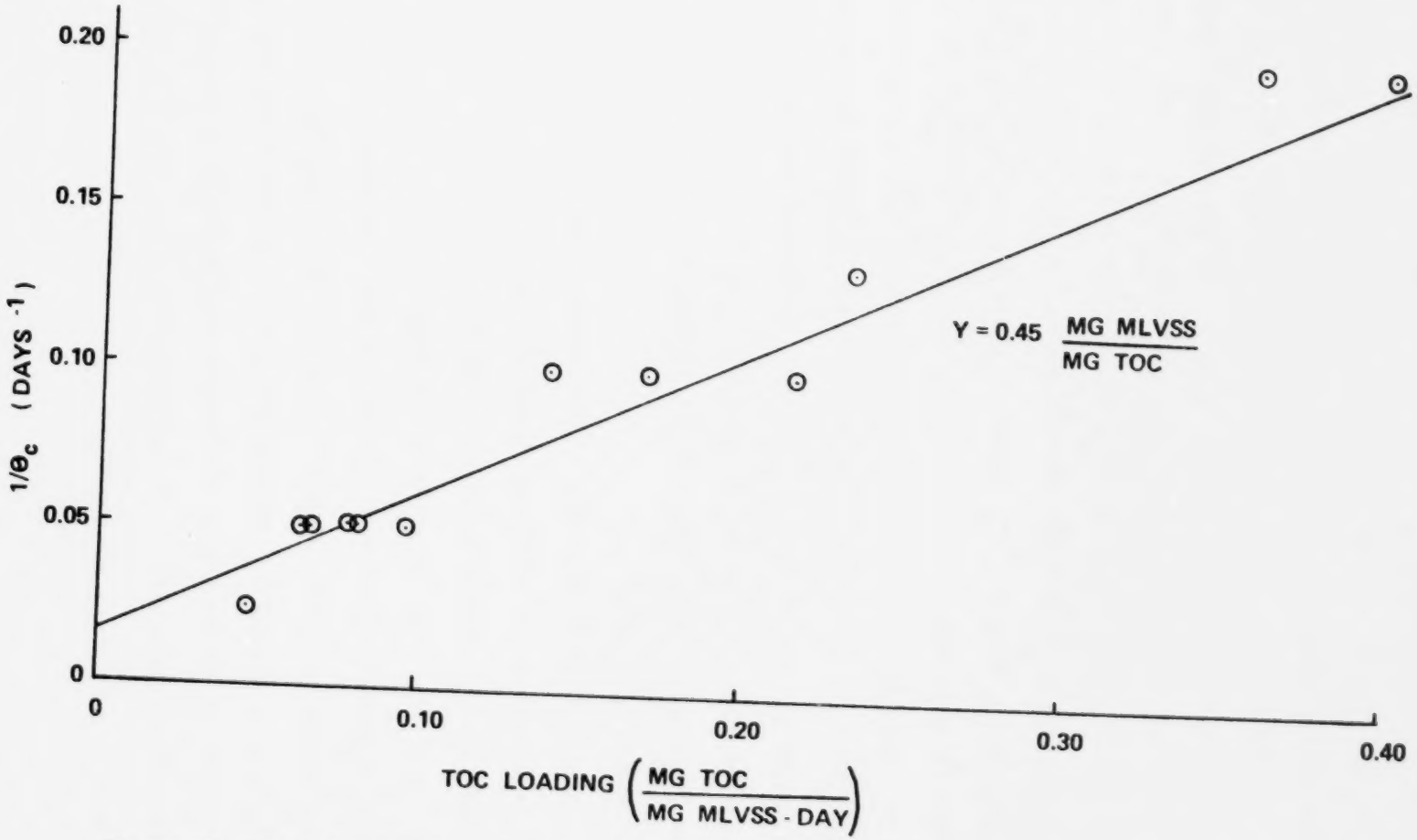


Figure 32. Relationship between solids residence time and TOC loading.

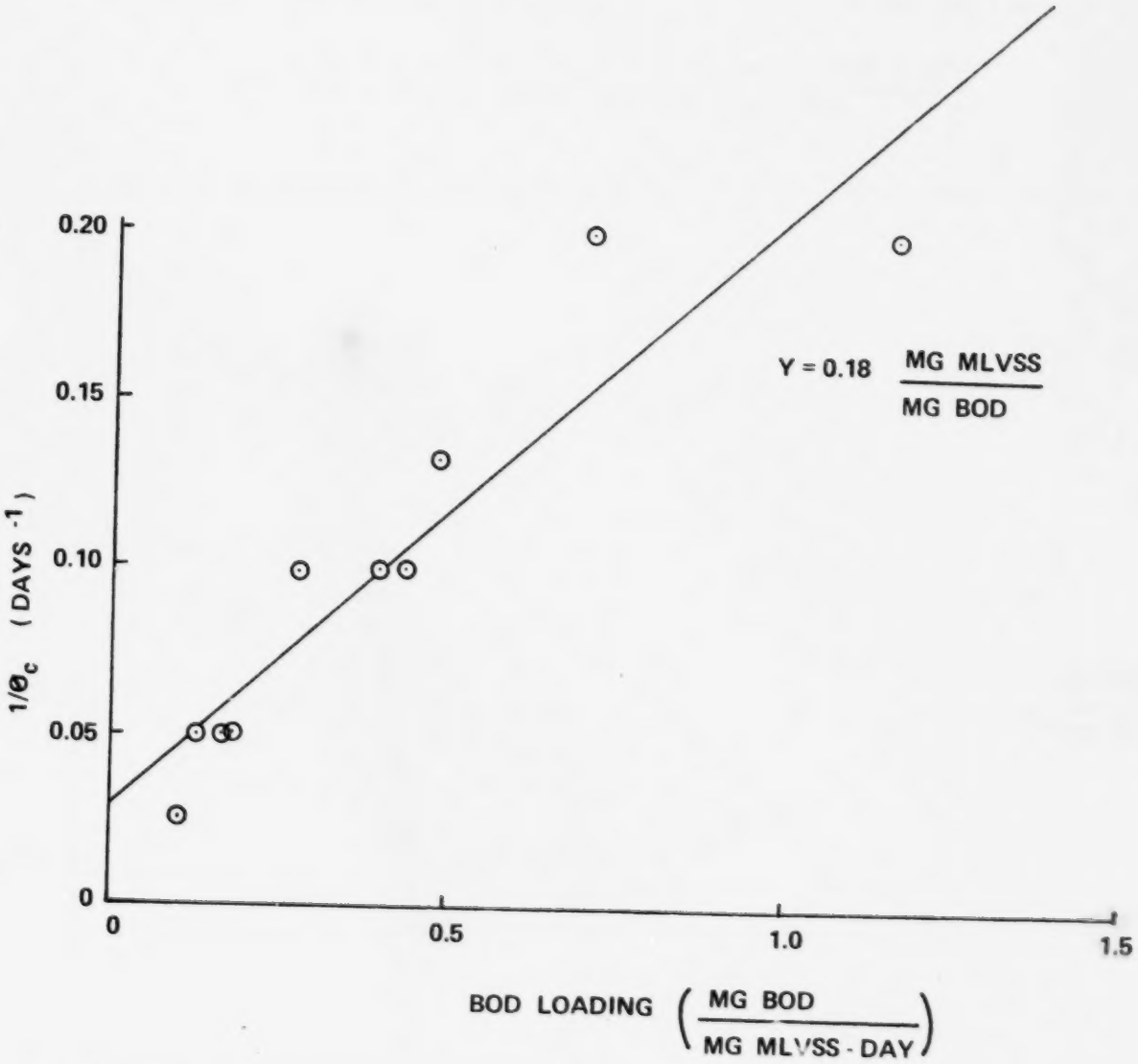


Figure 33. Relationship between solids residence time and BOD loading.

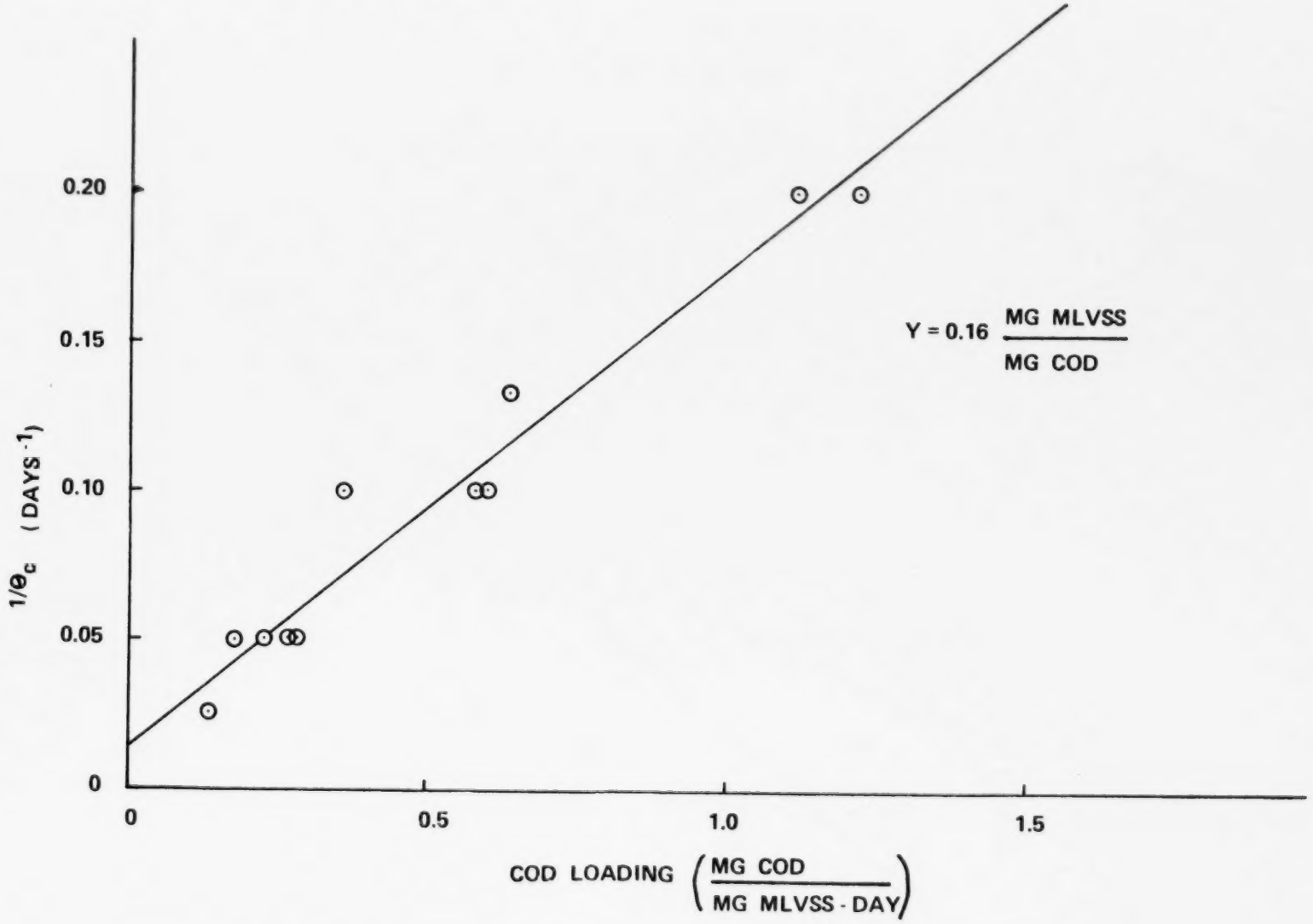


Figure 34. Relationship between solids residence time and COD loading.

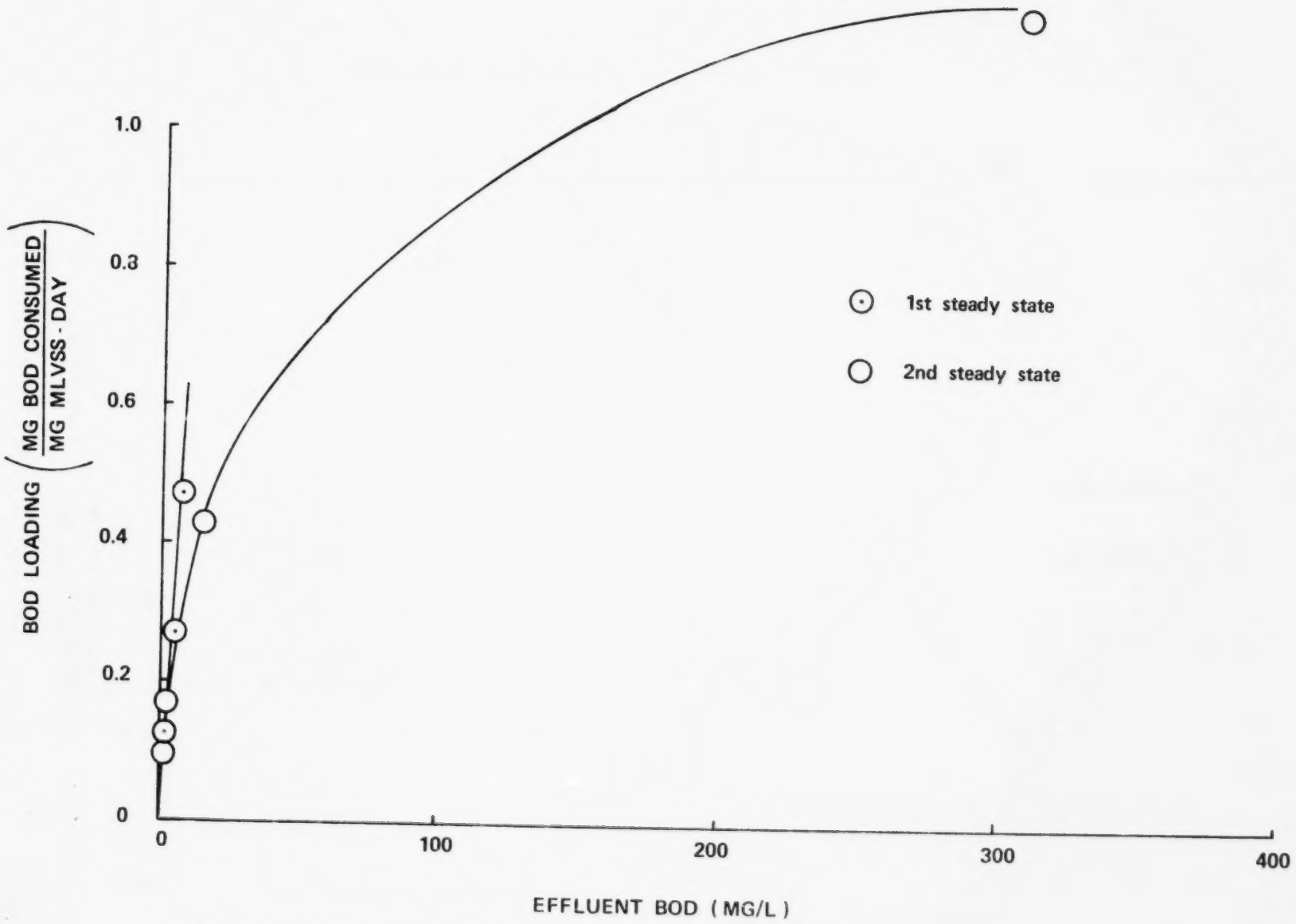


Figure 35. Kinetics of substrate utilization: BOD-basis.

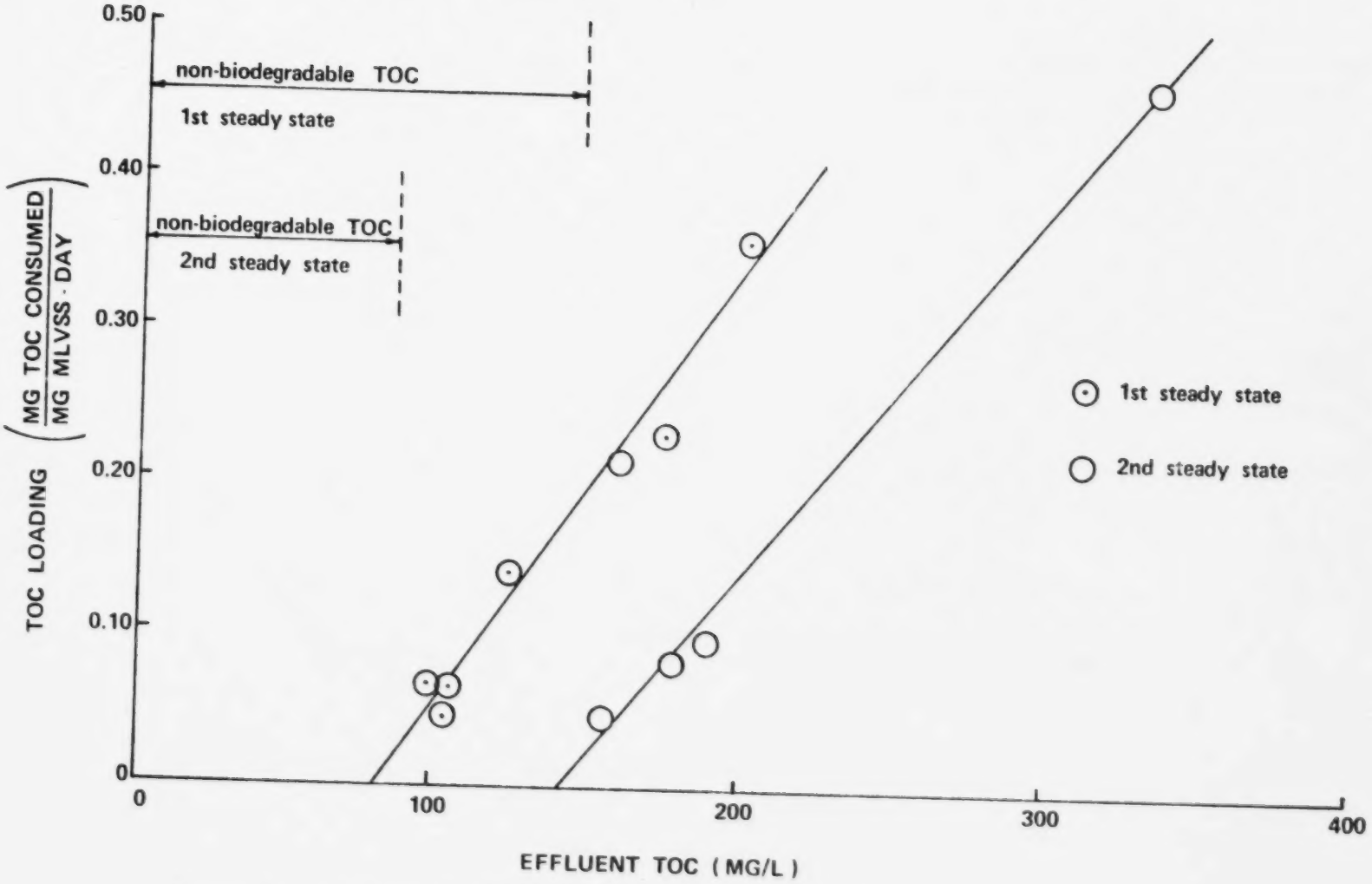


Figure 36. Kinetics of substrate utilization: TOC-basis.

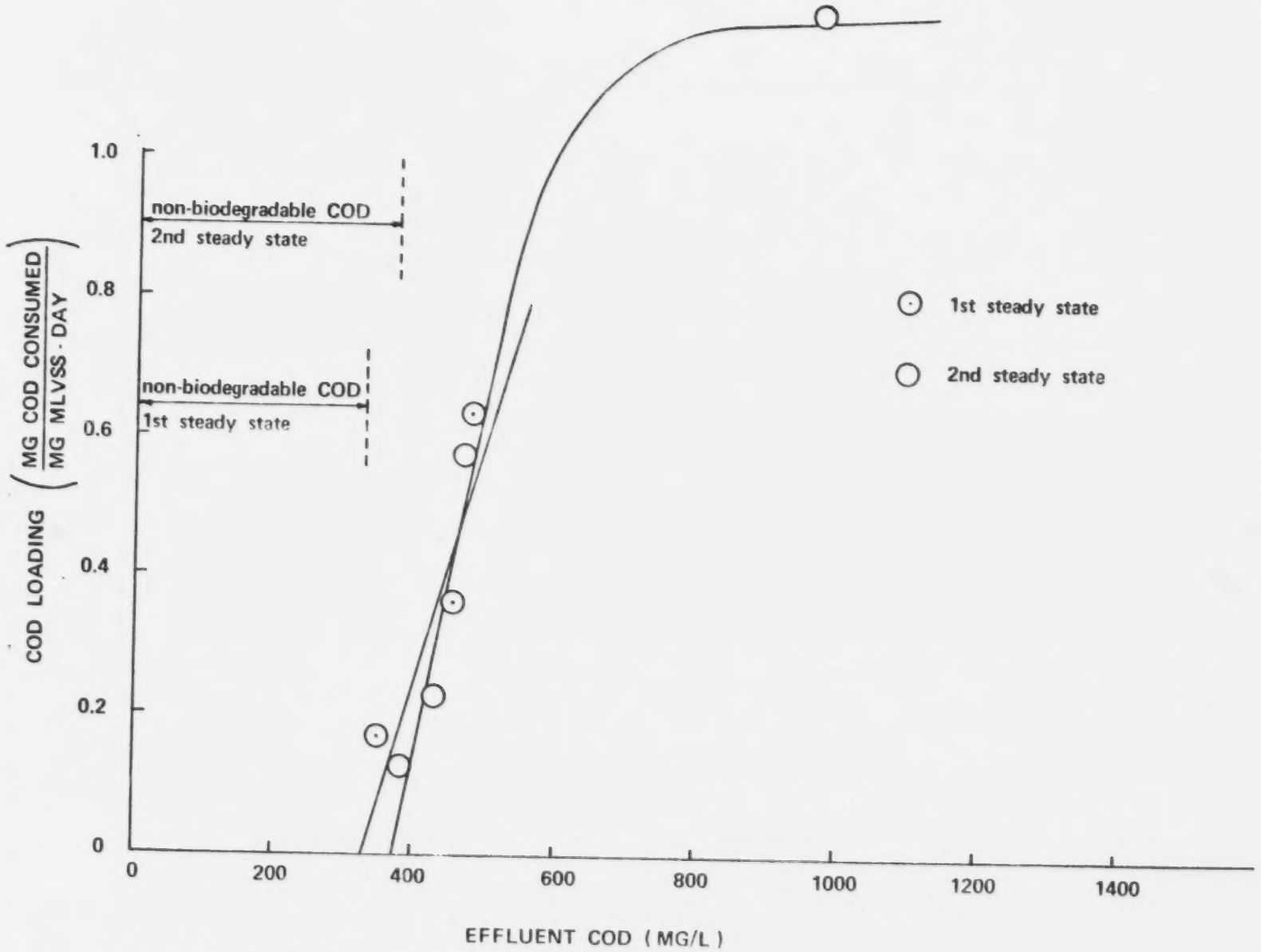
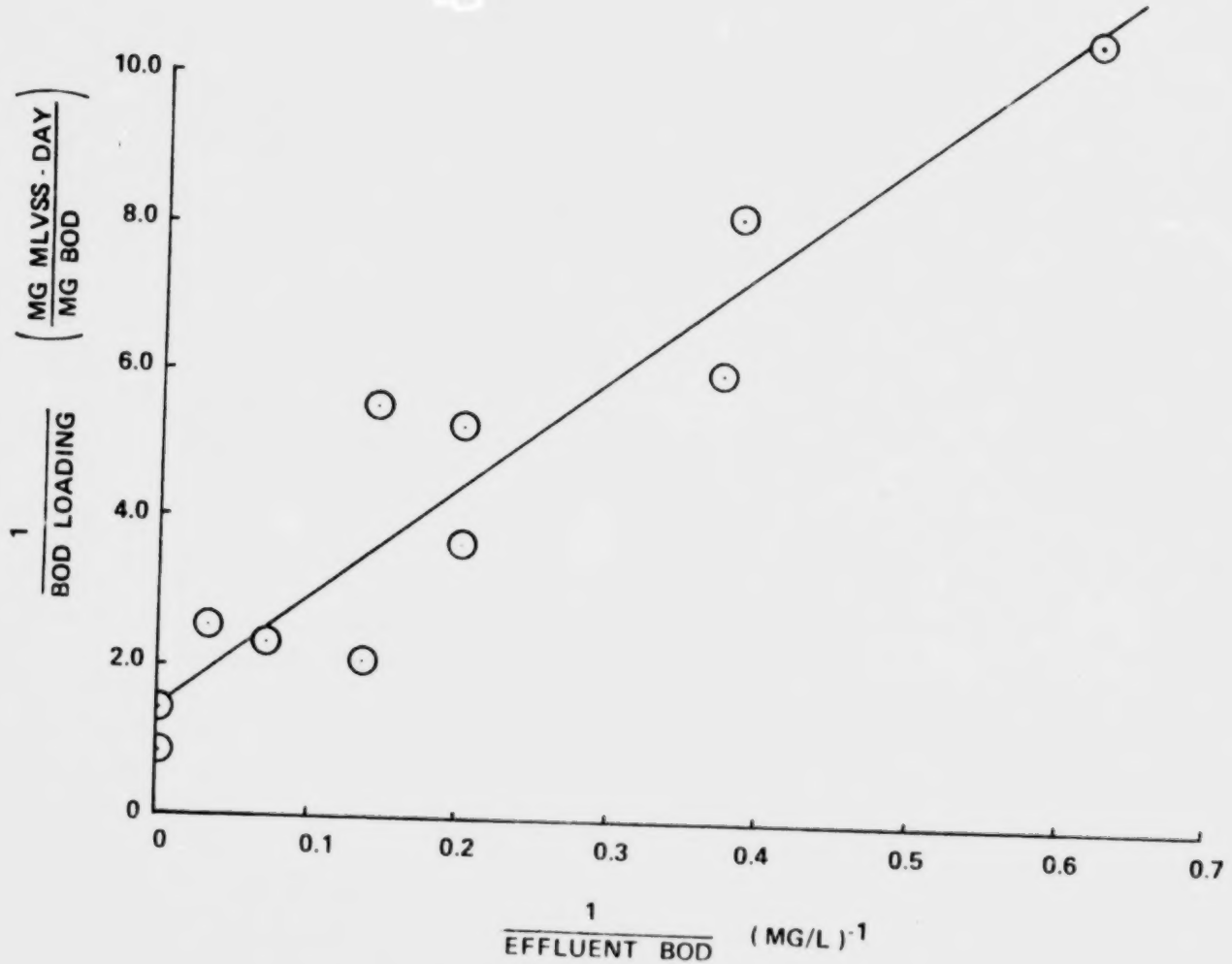


Figure 37. Kinetics of substrate utilization: COD-basis.

Figure 38. Linearized substrate utilization plot: BOD-basis.



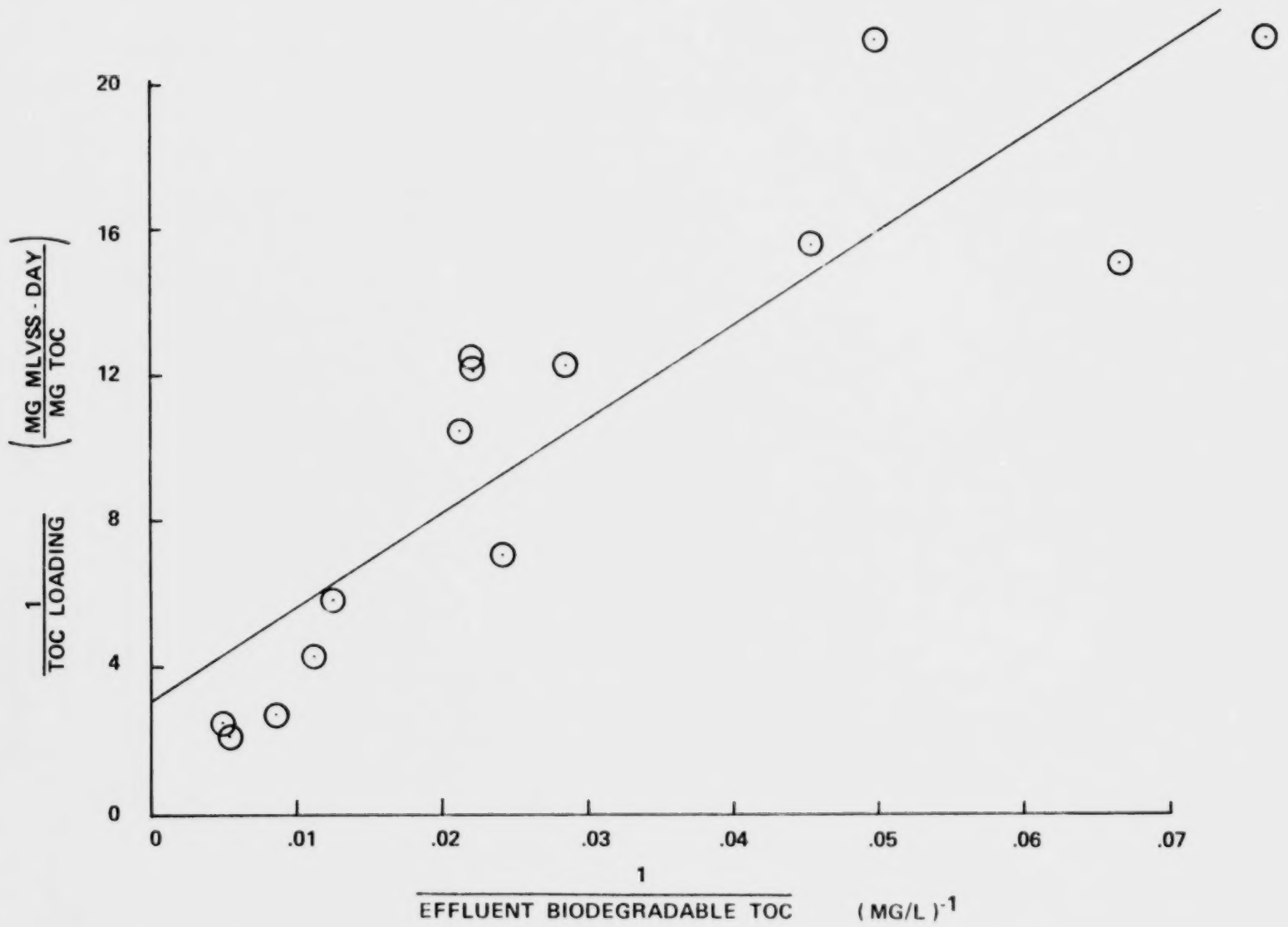


Figure 39. Linearized substrate utilization plot: TOC-basis.

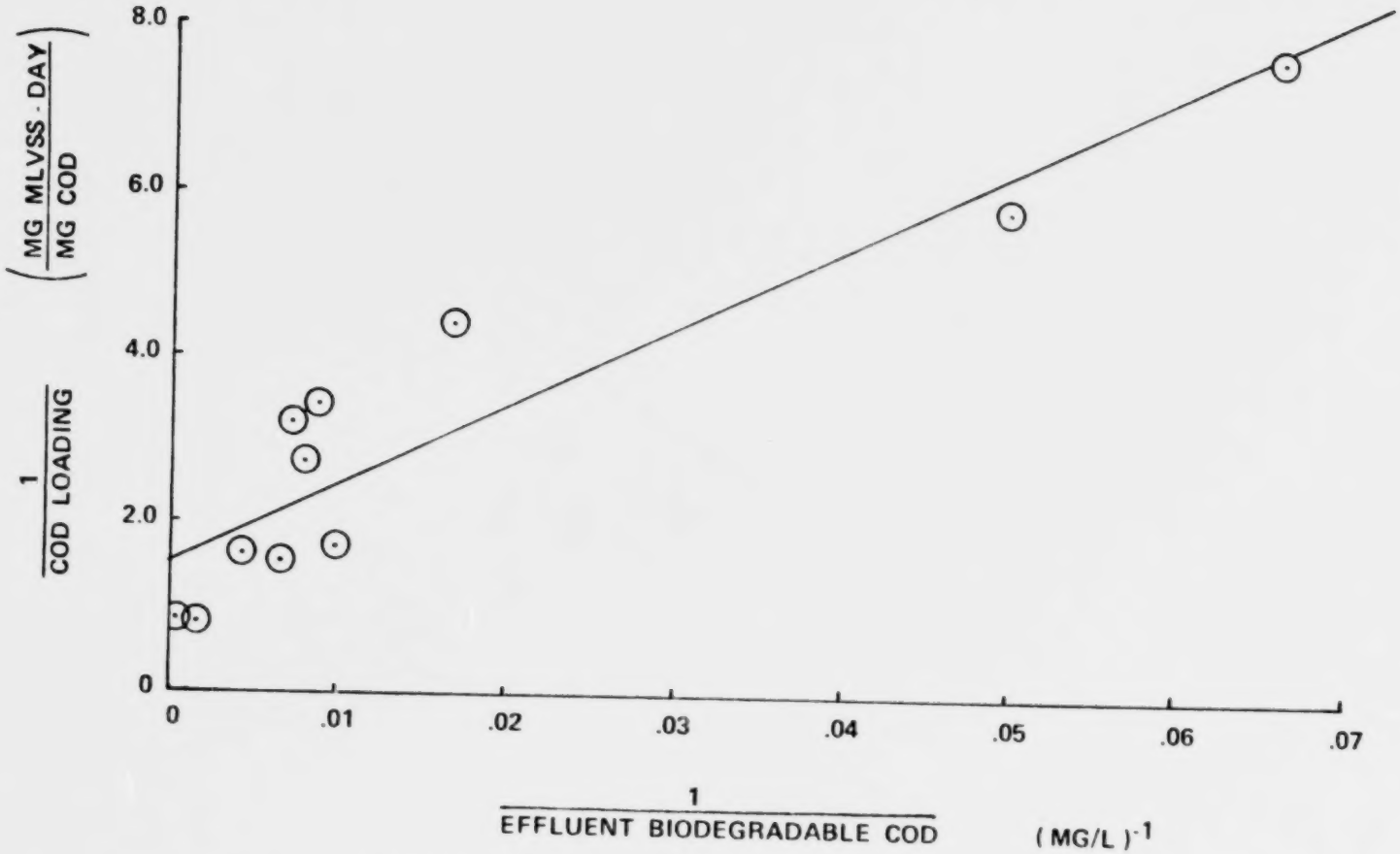


Figure 40. Linearized substrate utilization plot: COD-basis.

$$(S/U) = 1/k\ (S) + K_s/k \quad (7)$$

and

$$(U) = -K_s\ (U/S) + k \quad (8)$$

Table 15 lists the range of values for the specific substrate utilization rates and the Michaelis-Menten coefficients, determined from the slopes and intercepts of these different forms of linear plots. Also shown are the calculated yield coefficients as determined earlier from Figures 32 to 34.

It should be noted that the values reported here for the specific substrate utilization rate, are relatively low when compared to domestic wastewater and many other industrial wastes, reflecting the slow kinetics of the biochemical oxidation for phenolic wastewaters. The Michaelis-Menten coefficients, however, are in the same range as those reported for many other wastewaters (9).

TABLE 15. SUMMARY OF KINETIC COEFFICIENTS

Substrate Basis	Specific Substrate Utilization Rate, k mg substrate/mg MLVSS-day	Michaelis-Menten Coefficient, K_s mg/l	Microbial Yield, Y mg MLVSS/mg substrate
TOC	0.33 - 1.4	84 - 460	0.45
BOD	0.67 - 0.74	5.6 - 10.0	0.18
COD	0.64 - 1.3	60 - 240	0.16

Some of these values in Table 15 can be compared with those of other investigators for other types of coal conversion wastewaters. The COD yield coefficient is in the same range as those reported by Luthy and Tallon (3) for full-strength, ammonia-stripped and for diluted Hygas wastewater (y = 0.11 and 0.22, respectively). Their reported microbial die-away coefficient was 0.02 day^{-1}. On a BOD basis, Reap et al. (5) reported a yield of 0.48 mg VSS/mg BOD and a die-away coefficient of 0.03 day^{-1} for air-stripped H-Coal process wastewater. Their yield is significantly higher than that reported here.

Table 16 lists the substrate utilization and microbial growth kinetic coefficients determined by Drummond et al. (12) for the biological treatment of Synthane and Synthoil wastewater. The specific substrate utilization rates and the Michaelis Menten coefficients for the Synthane wastewater are quite comparable to ours, but the microbial yields are appreciably higher. The kinetic coefficients for the Synthoil wastewater are stated to be preliminary , and are presented here for illustrative purposes only.

The summary results reported in Table 15 should be considered only as preliminary results at this time and should not be used for design purposes. Biological treatability investigations are continuing and it is anticipated that these further investigations will lead to firmer criteria for designing biological treatment processes for coal conversion wastewaters.

TABLE 16. KINETIC COEFFICIENTS FROM BIOLOGICAL TREATMENT OF SYNTHANE AND SYNTHOIL WASTEWATER*

Substrate Basis	k mg substrate/mg MLVSS-day	K_s mg/l	Y mg MLVSS/mg substrate	K_d day^{-1}
A. Synthane Coal Gasification Wastewater				
TOC	0.49	100	1.1	0.04
BOD	0.46	5	0.53	0.02
COD	0.96	120	0.47	0.05
Phenol	0.63	30	--	--
B. Synthoil Coal Liquefaction Wastewater+				
TOC	0.14	3	1.2	0.004
BOD	0.39	14	0.61	0.02
COD	0.43	10	0.36	0.004
Phenol	--	--	--	--

*After Drummond et al. (12)

+Preliminary data only

SECTION 7

SPECIFIC ORGANIC ANALYSIS AND ENVIRONMENTAL ASSESSMENT OF TREATED WASTEWATERS: PRELIMINARY RESULTS

Raw wastewater and treated effluent from the biological reactors were collected at various times during the first two periods of study and subjected to specific organic analysis by high performance liquid chromatography (HPLC) and gas chromatography/mass spectrometry (GC/MS). Only the results for HPLC analysis are available at this time. Aquatic bioassays involving algae, Daphnia, and fish, and mammalian cytotoxicity analyses were also conducted on the samples as a means of assessing the aquatic and health impacts, respectively, of the biologically treated wastewater. This section presents some of the preliminary results from these specific organic and environmental assessment analyses.

HPLC ANALYSIS

Fresh samples of the reactor effluent were collected, filtered through 0.7 µm glass fiber filters, and injected into the HPLC. Care was taken to insure that the samples were taken during stable periods of reactor operation. Separation of the wastewater components in the samples was achieved on the HPLC using a 60-minute water/acetonitrile solvent gradient on a Waters µBondapak C_{18} analytical column. The eluted compounds were

detected by both UV absorbance at 280 nm and fluorescence at 275 nm excitation and 310 nm emission wavelengths.

Figure 41 shows chromatograms of the raw feed using both UV absorbance and fluorescence detectors. These chromatograms were used for purposes of compound identification. The numbered peaks correspond to the compounds listed in Table 17; elution volumes of the constituents are also shown. It should be noted that polarity decreases with increasing elution volume so that the elution volumes corresponding to the chromatographic peaks can be correlated to the polarity of the various organic compounds in the mixture. It should also be noted that each peak may represent more than one compound.

Figures 42 to 46 present chromatograms of the effluent from the 5-, 7.5-, 10-, 20- and 40-day reactors collected during the second series of reactor operation (see Section 5). The UV absorbance chromatograms of the reactor effluents were all prepared at the same sensitivity (x10) and can be compared directly; the chromatogram for the raw feed is at a lower sensitivity (x5). The fluorescence chromatograms should be observed carefully as they were each prepared at different detector sensitivities.

The UV chromatograms of the reactor effluents reflect the production of highly polar compounds (e.g. aliphatic and aromatic acids, etc.) as a result of biological treatment. The concentration of these polar compounds, which appear at low and intermediate elution volumes, does not appear to change with increasing degree of treatment, i.e. detention time. Some of these peaks may be attributed to the production of aliphatic acids which can be expected from the bacterial degradation of phenolic compounds.

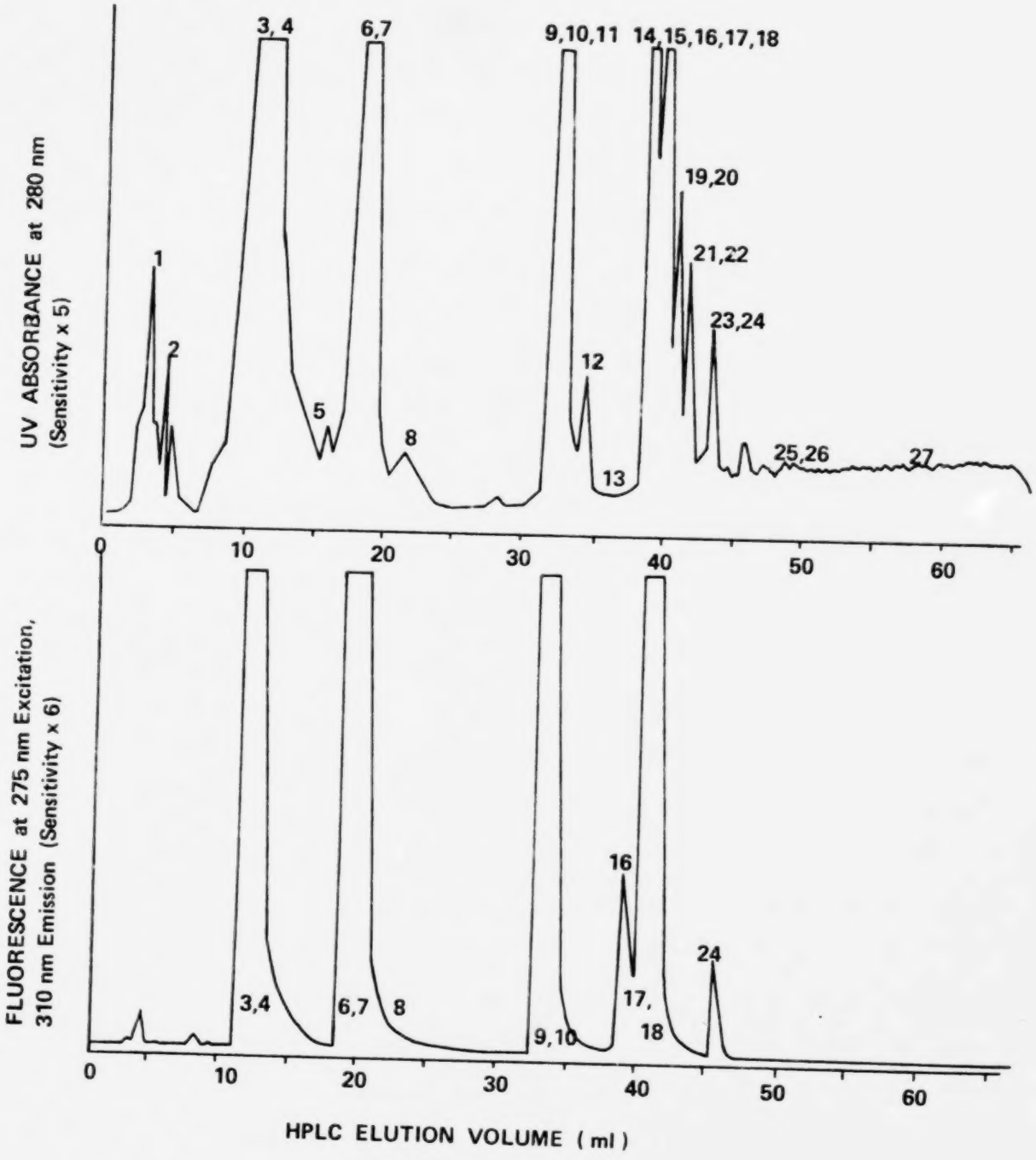


Figure 41. HPLC chromatographic profiles of raw synthetic wastewater. (Numbers correspond to compounds in Table 17.)

TABLE 17. IDENTIFICATION OF HPLC PEAKS

Peak Number	Elution Volume, ml	Compound Name
1	3	acetic, hexanoic, benzoic acids
2	4	solvent
3	13	resorcinol
4	14	catechol
5	18	aniline
6	21	phenol
7	22	5-methylresorcinol
8	23	4-methylcatechol
9	33	p-cresol
10	33	o-cresol
11	34	2-indanol
12	35	acetophenone
13	28	4-ethylpyridine
14	40	pyridine
15	40	quinoline
16	40	3,5-xylenol
17	41	3,4-xylenol
18	41	2,3-xylenol
19	42	indole
20	42	3-ethylphenol
21	43	2-methylquinoline
22	43	1-naphthol
23	45	2-naphthol
24	45	2,3,5-trimethylphenol
25	50	indene
26	51	naphthalene
27	58	anthracene

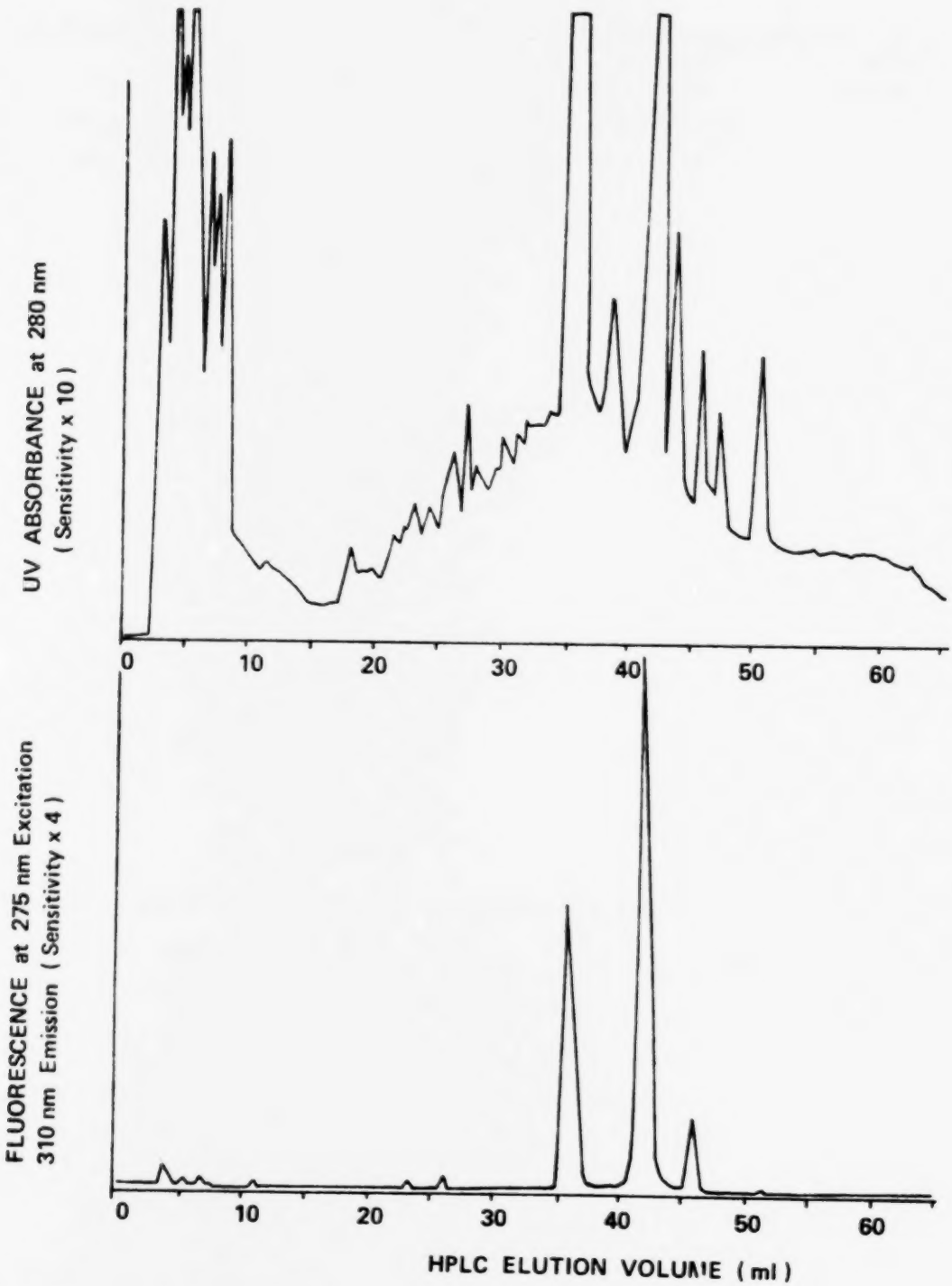


Figure 42. HPLC chromatographic profiles of 5-day reactor effluent.

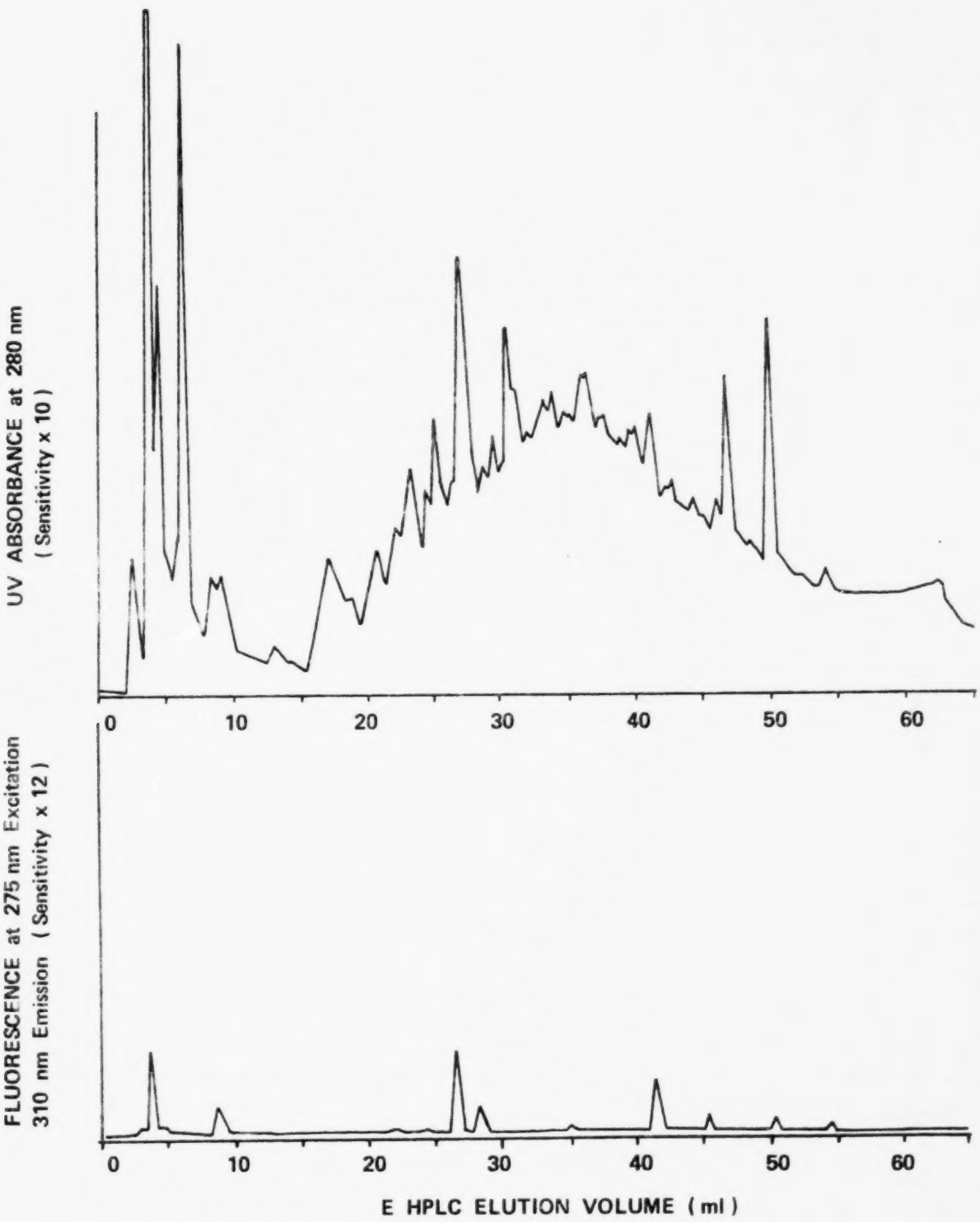


Figure 43. HPLC chromatographic profiles of 7.5-day reactor effluent.

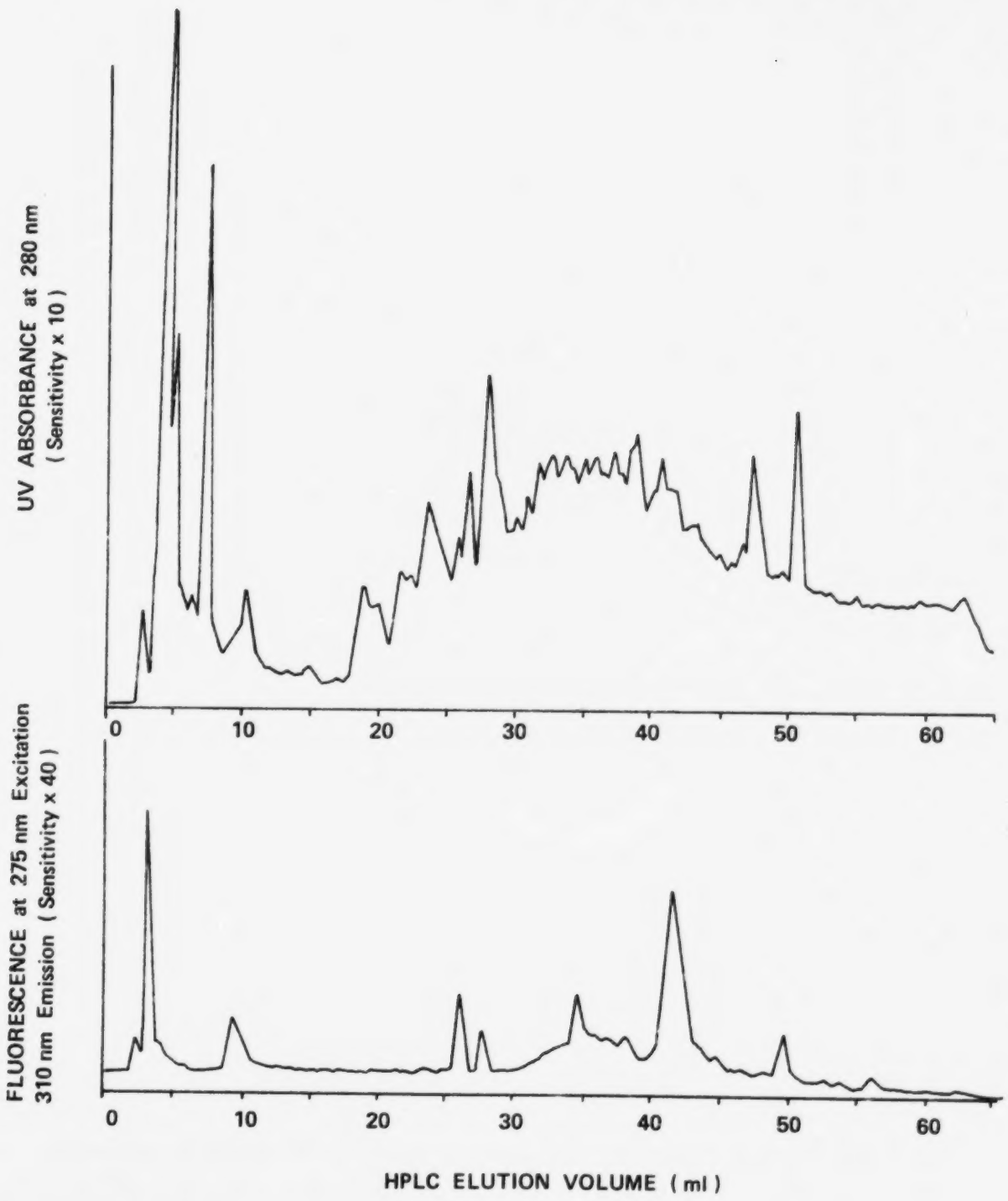


Figure 44. HPLC chromatographic profiles of 10-day reactor effluent.

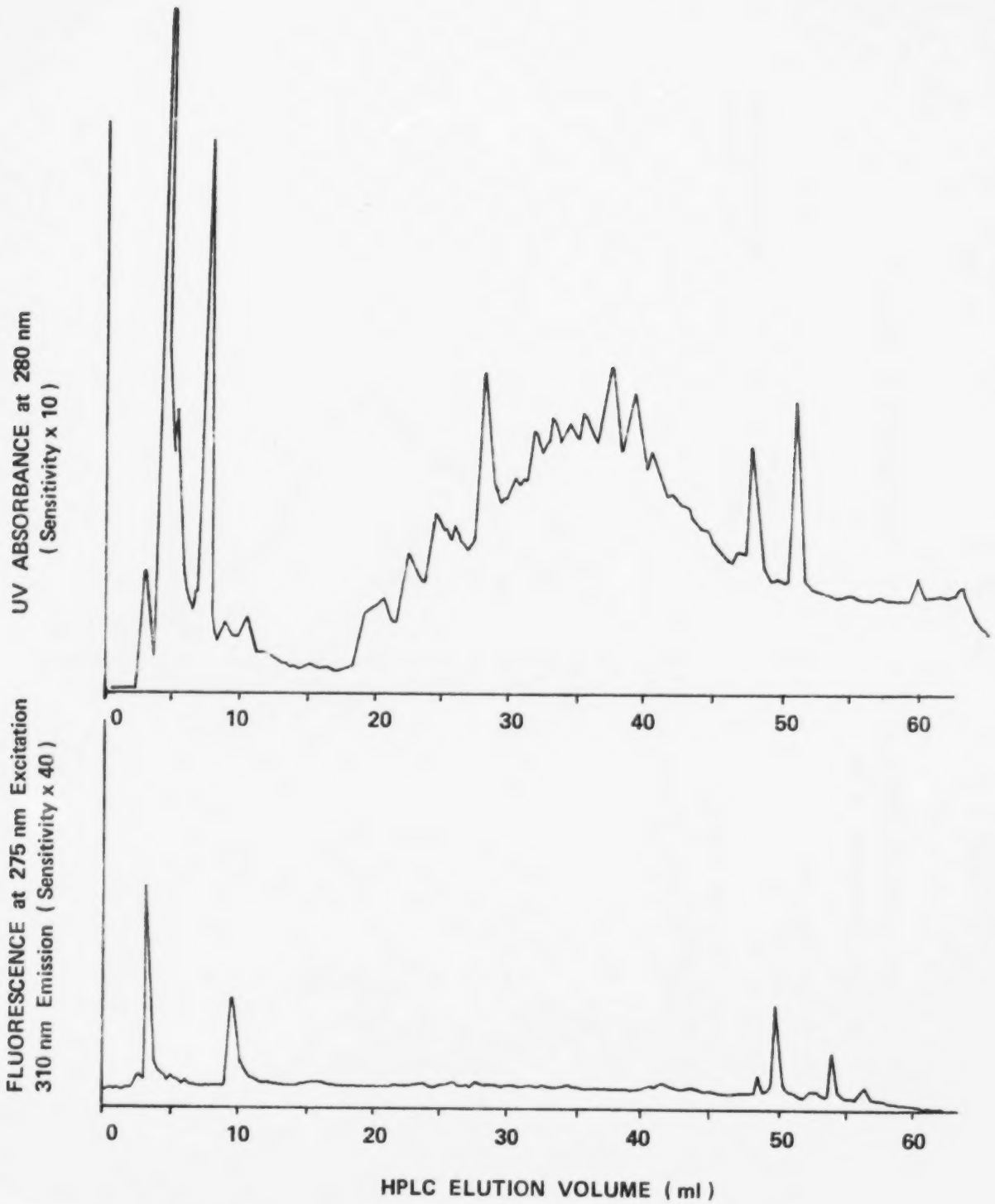


Figure 45. HPLC chromatographic profiles of 20-day reactor effluent.

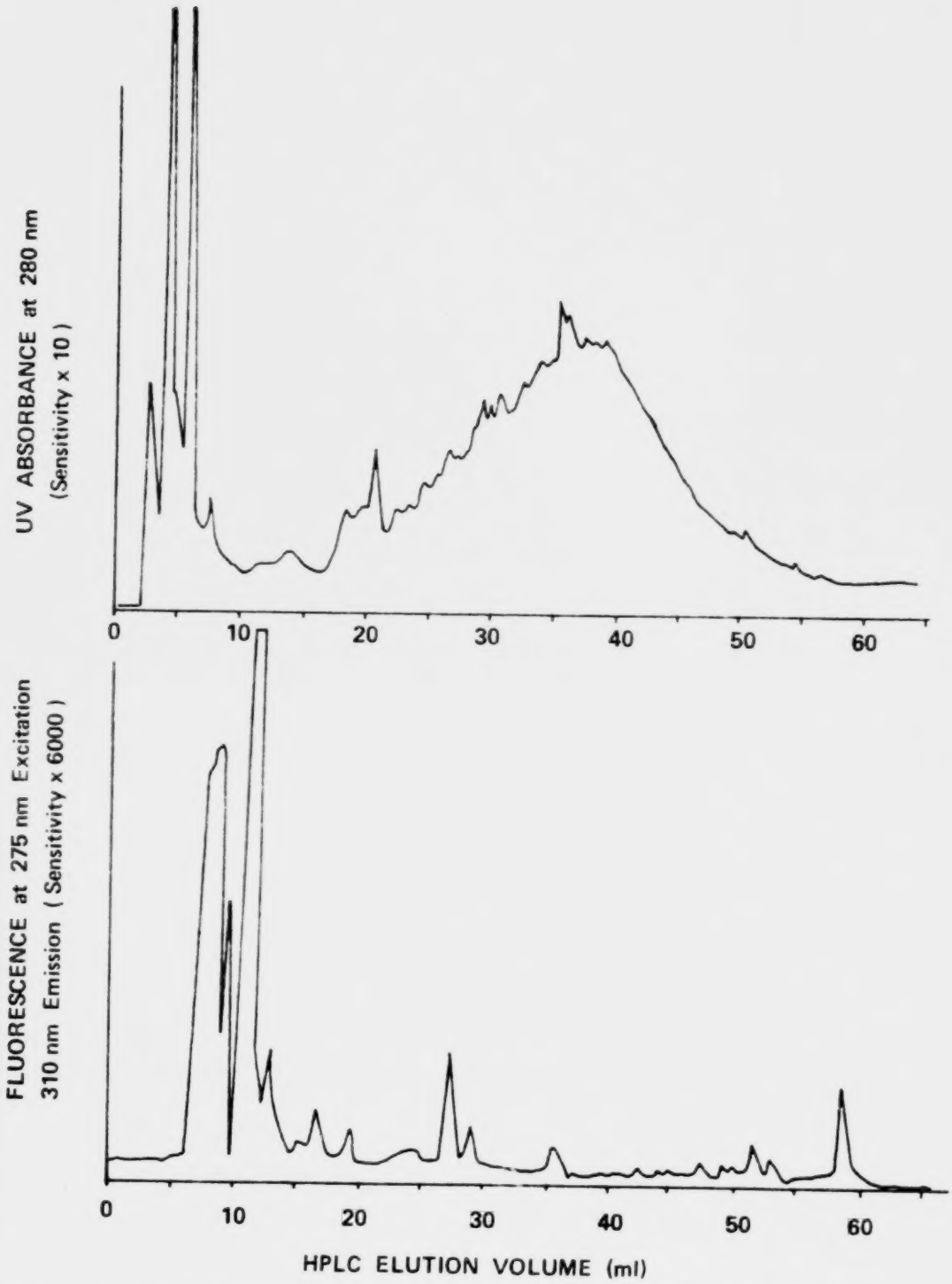


Figure 46. HPLC chromatographic profiles of 40-day reactor effluent.

On the other end of the UV chromatograms (at the high elution volumes), a greater reduction of the more non-polar compounds (e.g., pyridine, quinoline, xylenols, ethylphenols, trimethylphenol and naphthols) can be seen with increased reactor detention time. This is especially true for the reactors with detention times greater than 5 days, where the chromatographic peaks for these compounds were too small to detect among the peaks for the cellular metabolites produced during biological treatment. (It should be noted that direct probe mass spectral analysis indicates that the two significant UV peaks at elution volumes of 47 and 51 mls are phthalic acid esters, possibly arising from the tygon tubing or plexiglass used in construction and operation of the biological reactors.)

HPLC traces of the reactor effluents were used to obtain approximate concentrations for several of the major constituents in the raw feed to the reactors. In view of the selectivity of the fluorescence wavelengths used for phenolic compounds and the complexity of the UV chromatograms due to the presence of large quantities of UV-absorbing cellular by-products in the reactor effluents, all quantitation of phenolics was accomplished using fluorescence detection. It should be noted that the elution volume at which a given peak occurs may vary slightly from one chromatogram to the next depending upon column age, sample volume, and sample concentration. Hence, quantitation of the compounds of interest was performed by preparing several chromatograms with standard additions of these compounds. An illustrative chromatogram of this "spiking" technique is shown in Figure 47. The concentrations of the major phenolic compounds in each of the reactor effluents, as determined in this manner, are listed in Table 18. The concentrations in some cases are reported as less than a certain value

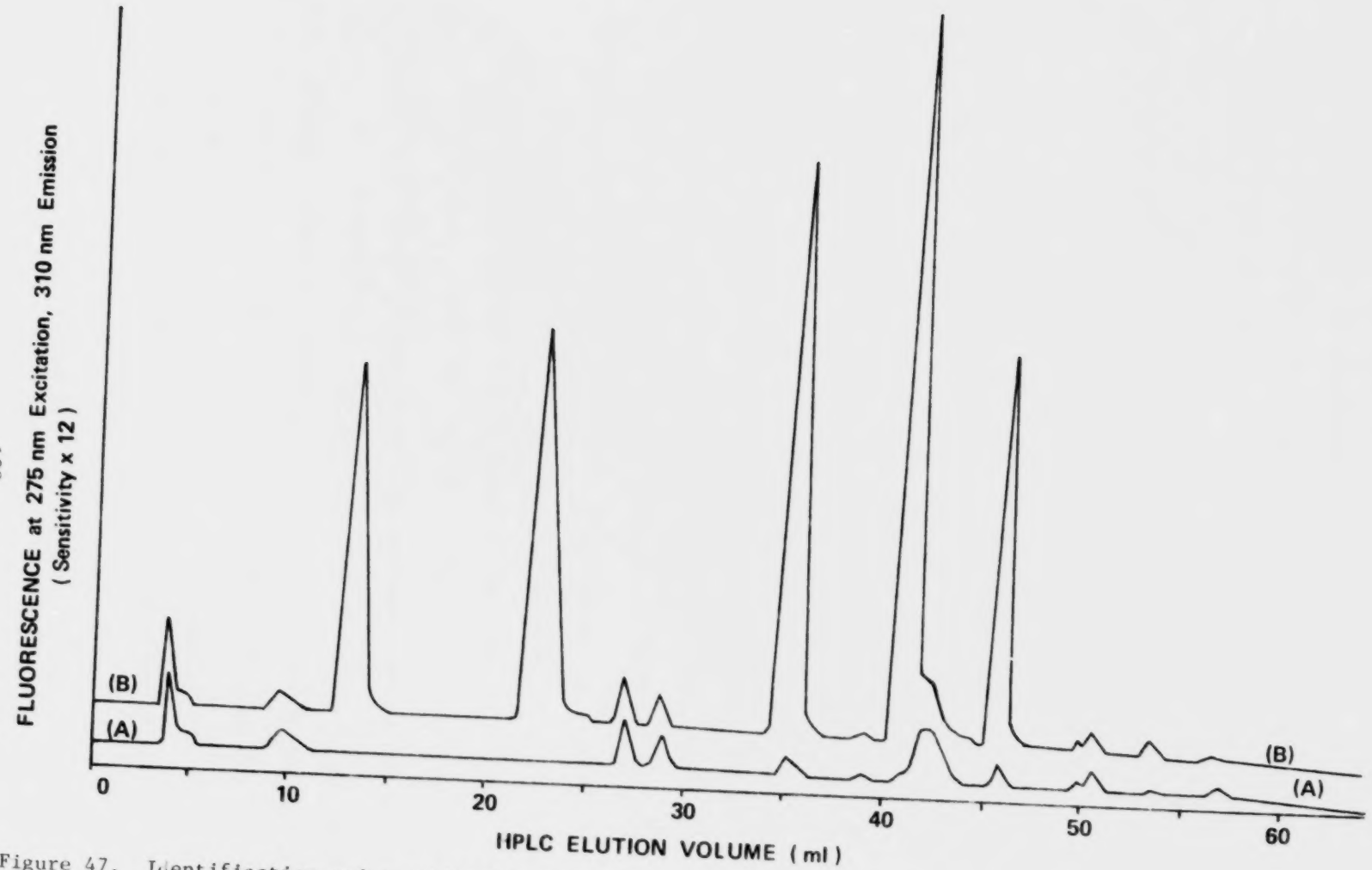


Figure 47. Identification and quantitation of HPLC chromatographic peaks from 10-day reactor. (A.) Chromatogram of 10-day reactor effluent. (B.) Chromatogram of 10-day reactor effluent spiked with 20 mg/l resorcinol, 20 mg/l phenol, 10 mg/l p-cresol, 10 mg/l 3,4-xylenol, and 10 mg/l 2,3,5-trimethylphenol.

TABLE 18. CONCENTRATIONS OF MAJOR PHENOLIC COMPOUNDS IN REACTOR EFFLUENTS (mg/l)

Compound	Raw Feed (1/4-strength)	5-day reactor 4/12/79	5-day reactor 4/24/79	7.5-day reactor 5/7/79	10-day reactor 4/25/79	20-day reactor 4/25/79	20-day reactor 5/4/79	40-day reactor 8/30/79
catechol	250	<0.5	<0.5	<0.2	<0.5	<0.2	<0.1	<0.02
resorcinol	250	<0.5	<0.5	<0.2	<0.5	<0.2	<0.1	<0.02
phenol	500	0.9	0.6	<0.2	<0.4	<0.2	<0.1	<0.13
cresols	162.5	22.2	30.2	0.2	0.8	<0.005	<0.02	0.036
o-cresol	100							
p-cresol	62.5							
xylenols	135							
3,4-xylenol	62.5	33.6	31.4	1.0	2.5	1.4	<0.01	0.007
2,3-xylenol	62.5							
3,5-xylenol	10.0							
2,3,5-trimethyl-phenol	12.5	9.0	7.0	0.6	1.3	<0.08	<0.02	<0.004

where that value represents the detection limit of the HPLC fluorescence detector for that compound at the sensitivity used for the analysis. The table shows that removal of the phenolics increases with increased detention time and that phenol, resorcinol and catechol are almost completely removed by the 5-day reactor. The cresols are completely removed within 7.5 to 10 days (to concentrations less than 1 mg/l) while a 20 day sludge age is required to reduce the concentrations of the xylenols and trimethylphenol below 1 mg/l. These observations are in accordance with the phenol data using the aminoantipyrine wet chemical procedure reported earlier in Section 5, and with the biodegradability results in Section 9. These results are significant from the standpoint of reactor performance since a large portion of the organic carbon in the influent feed is comprised of phenolic compounds.

Analysis of the reactor effluents is continuing using HPLC and other fluorescence wavelengths, gas chromatography/ mass spectrometry, and direct ion probe mass spectrometry on HPLC-separated fractions. These results should be available in the next report in this series.

AQUATIC BIOASSAY

Traditionally, short-term lethality tests with fish have received greatest emphasis in developing and evaluating pollution abatement programs. More recently, the realization that elimination of lower organisms may have serious environmental consequences has led to increased reliance on algae and invertebrate assays as well. Several aquatic organisms, including the fathead minnow (Pimephales promelas), a cladoceran (Daphnia pulex), and an alga (Selenastrum capricornutum) have become widely

accepted bioassay organisms, and routine bioassay procedures are available (8, 13).

In this section, results of some preliminary bioassay experiments performed with fathead minnows, D. pulex, and S. capricornutum exposed to raw and treated synthetic coal conversion wastewaters are presented. Although the preliminary findings suggest that toxicity of the raw wastewater is substantially reduced following biological treatment, the present data are not definitive. However, they provide basic information regarding: a) sample collection and handling of synthetic effluents for conducting bioassays; b) procedural modifications of bioassay methods necessary to accommodate toxicity data interpretation; and c) formulation of appropriate experimental protocols for more detailed analyses of acute toxicity problems associated with coal conversion wastewaters.

In the preliminary studies presented herein, no attempt was made analytically to verify actual effluent concentrations from the biotreatability reactors nor to monitor possible loss of TOC through volatilization or biodegradation during the course of the bioassays. Rather, nominal concentrations of the effluents were prepared by serial dilution procedures using volumetric glassware.

The raw synthetic wastewater for toxicity tests was drawn from the reservoir feeding the bioreactor pilot units. All samples were drawn from freshly-made synthetic wastewater, from 2 to 10 hours after preparation. If the raw feed was not to be used immediately, aliquots were frozen and stored until needed. Effluents from the 5-, 10-, and 20-day biological reactors were collected from glass reservoirs which contained the overflow from the pilot units. Prior to sample collection each day, the reservoirs

were emptied to ensure that the respective effluents were less than 24 hours old. The reactor effluents were centrifuged, and the resultant supernatant was filtered through glass wool to remove suspended solids. Filtrates were frozen in 1 to 3 liter aliquots for the Daphnia and Selenastrum toxicity tests. However, due to the large sample volumes required for tests with the fathead minnows, daily collections were centrifuged, filtered, and frozen in 5-gallon soft glass jars, until composite effluent samples of 35-40 liters were accumulated. All samples subjected to the aquatic bioassay tests were collected during the second set of biotreatability studies described in Section 5.

General bioassay procedures for fathead minnows and Daphnia pulex were conducted in accordance with Standard Methods(8). Test concentrations of the effluents were prepared on a volume percent basis by dilution with carbon-filtered dechlorinated tap water. The dilution water had an average pH of 7.2, total alkalinity of 36.7 mg/l (as $CaCO_3$), total hardness of 30.8 mg/l (as $CaCO_3$), and total residual chlorine of 0.04 mg/l. All tests were conducted in a constant temperature laboratory at 18 to 20°C as static non-renewal bioassays. Two replicates, each containing 15 test organisms, were used for each effluent concentration. Sample volumes for each replicate were 10 liters and 100 ml, respectively, for fathead minnows and Daphnia. For the former, the duration of the test was 96 hours and death was used as the bioassay endpoint. For the latter, toxicity tests were conducted for 48 hours with immobilization as the endpoint. The effluent concentrations used in these preliminary experiments, as well as their respective effects on both test organisms, are summarized in Table 19.

TABLE 19. EFFECTS OF RAW AND BIOLOGICALLY-TREATED WASTEWATERS ON FATHEAD MINNOWS AND DAPHNIA PULEX

FATHEAD MINNOWS

RAW WASTEWATER

Concentration, % by volume	Percent Mortality
3.2	100
1.8	100
1.0	16.6
0.56	0.0
0.32	3.3
0.18	0.0
0.10	6.7

5-DAY REACTOR

Concentration % by volume	Percent Mortality
30	100
25	100
20	100
15	100
05	13.3
2.5	3.3

10-DAY REACTOR

Concentration % by volume	Percent Mortality
28	40.0
21	20.0
18	20.0
13.5	0.0
10.0	0.0
7.5	0.0
5.6	0.0
3.2	0.0

DAPHNIA PULEX

RAW WASTEWATER

Concentration, % by volume	Percent Immobilization
3.2	83.0
1.8	83.0
1.0	86.0
0.56	60.0
0.32	50.0

5-DAY REACTOR

Concentration % by volume	Percent Immobilization
10	73.3
5.6	50.0
3.2	33.3
1.8	16.7
1.0	0

10-DAY REACTOR

Concentration % by volume	Percent Immobilization
32	100
18	93.3
10	63.0
5.6	60.0
3.2	23.3

20-DAY REACTOR

Concentration % by Volume	Percent Immobilization
32	40
18	10
10	13.3
5.6	0.0
3.2	0.0

Although several algal assays have been initiated to examine the growth response of Selenastrum capricornutum exposed to both raw and treated synthetic coal conversion wastewater, the current discussion is limited to one range-finding test. This particular test was conducted to delineate effluent concentrations for more definitive toxicity analysis, and only one test flask was used for each concentration. However, the data are of interest in that they provide some basis of comparison between the raw feed and all three bioreactor effluents. In order to remove bacteria from the effluent samples prior to preparing the dilutions, all samples were filtered through 0.2 µm membrane filters. Raw feed concentrations of 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1.0, and 5.0%, as well as reactor effluent concentrations of 0.01, 0.05, 0.1, 0.5, 1.0. 5.0, 10.0, and 50.0% were inoculated with $1x10^3$ algal cells per ml, and incubated for 12 days. Growth curves for Selenastrum capricornutum cultures exposed to selected raw and treated wastewater concentrations of interest are shown in Figures 48 through 51.

The preliminary toxicity data for fish and Daphnia summarized in Table 19 do not meet appropriate assumptions for reliable estimation of LC_{50}'s over the concentration ranges tested. However, the dose-response curves constructed from these data for both fathead minnows (Figure 52) and Daphnia pulex (Figure 53) reflect an obvious trend of marked toxicity reduction of the synthetic wastewater following biological treatment. Further, the extent of toxicity reduction appears to be related to the degree of biological treatment as reflected by the solids residence times of the biological reactors. Considering the number of readily biodegradable compounds present in the raw feed, these results are not unexpected.

While growth data for Selenastrum capricornutum are admittedly limited, it is of interest to note that relatively low concentrations, of the raw and treated synthetic wastewaters either stimulated growth or were not significantly different from the controls. On the other hand, if the 1, 5, and 10% dilutions are compared for all the effluents tested (see Figures 48 to 51), toxicity again appears to be reduced to a greater extent with increasing reactor detention time. It should be pointed out, however, that several problems were encountered with both fungal and bacterial contamination of the algal cultures. In subsequent tests with the raw wastewater, it was found that after several days, bacterial populations in the test flasks began to proliferate. Therefore, the observed reduction in cell yield may have been due to inter-specific competition, as well as toxicity.

As the results are only of a preliminary nature, the effluents from each of the biological reactors will be studied more fully in the future, in order to provide definitive answers concerning acute toxicity of the wastewaters to the three aquatic organisms described above. Specific objectives to be considered include detailed characterization of the toxicity curve associated with the raw wastewater and reactor effluents, definitive assessment of reduced toxicity resulting from biological waste treatment, and potential loss of wastewater constituents through volatilization and biodegradation during the course of the bioassay. Further, studies are being initiated to ascertain whether continuous-flow toxicity tests will provide a more realistic impact assessment than static bioassays with fish.

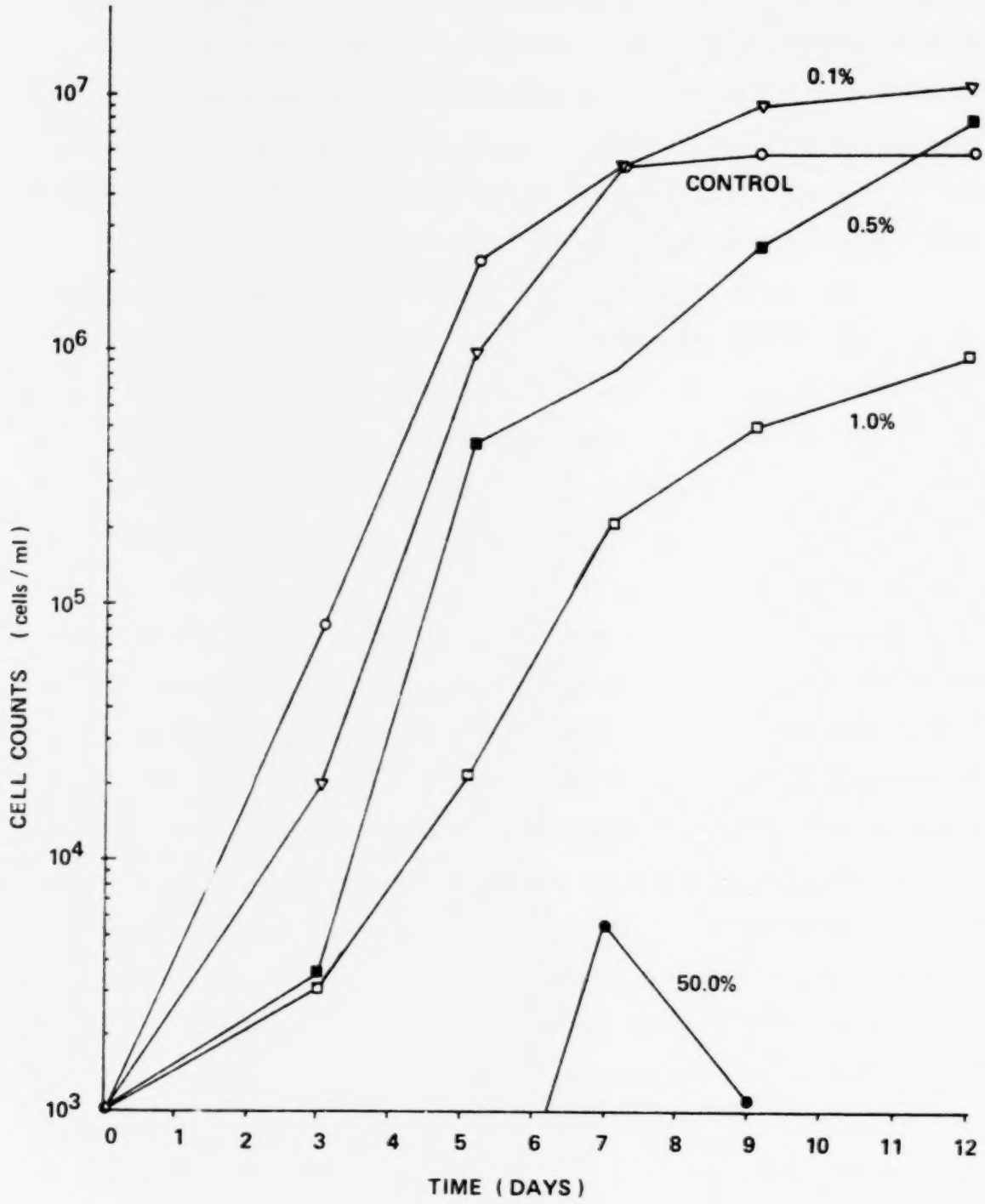


Figure 48. Growth of Selenastrum capricornutum exposed to various dilutions of raw synthetic wastewater.

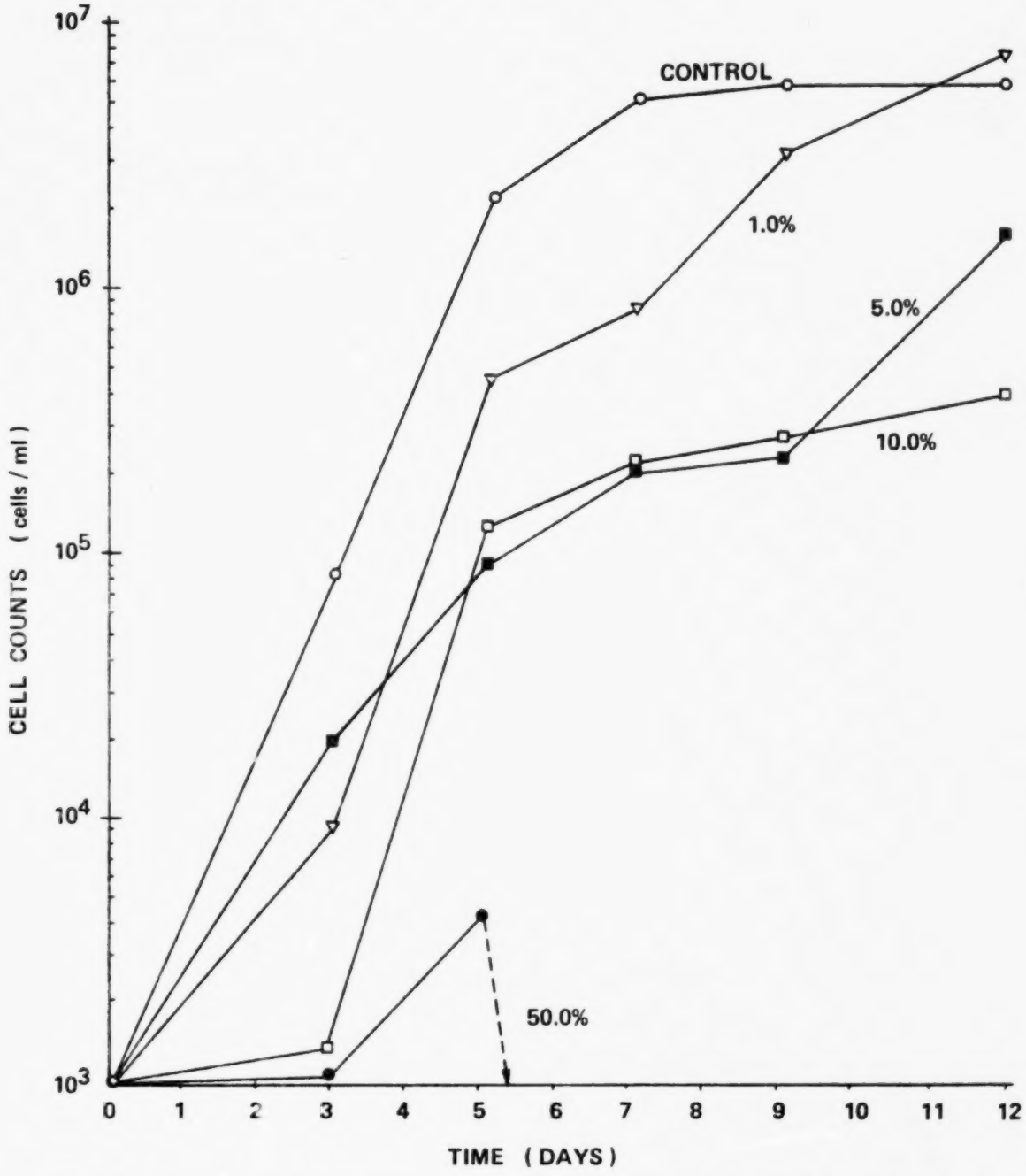


Figure 49. Growth of Selenastrum capricornutum exposed to various dilutions of 5-day reactor effluent.

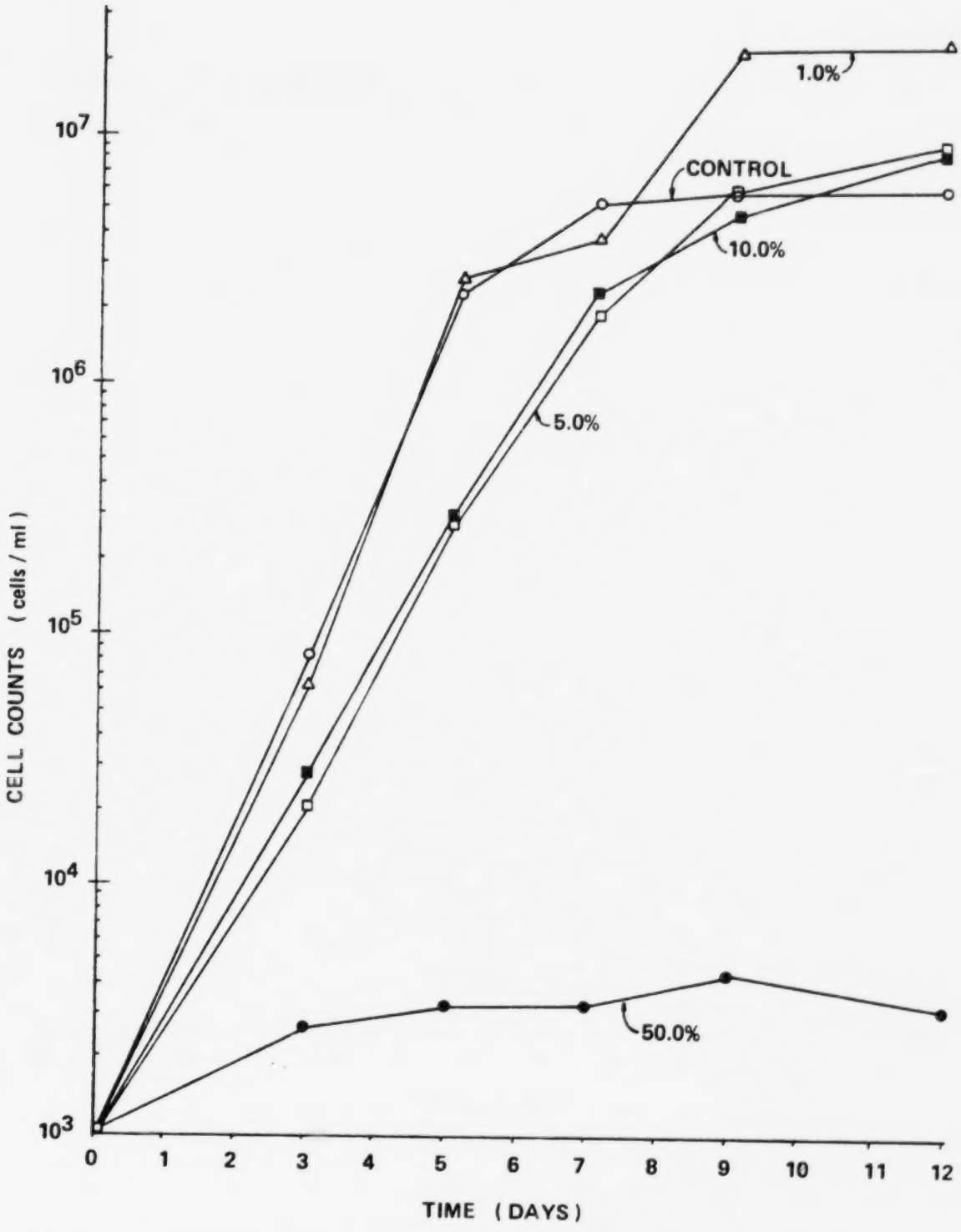


Figure 50. Growth of Selenastrum capricornutum exposed to various dilutions of 10-day reactor effluent.

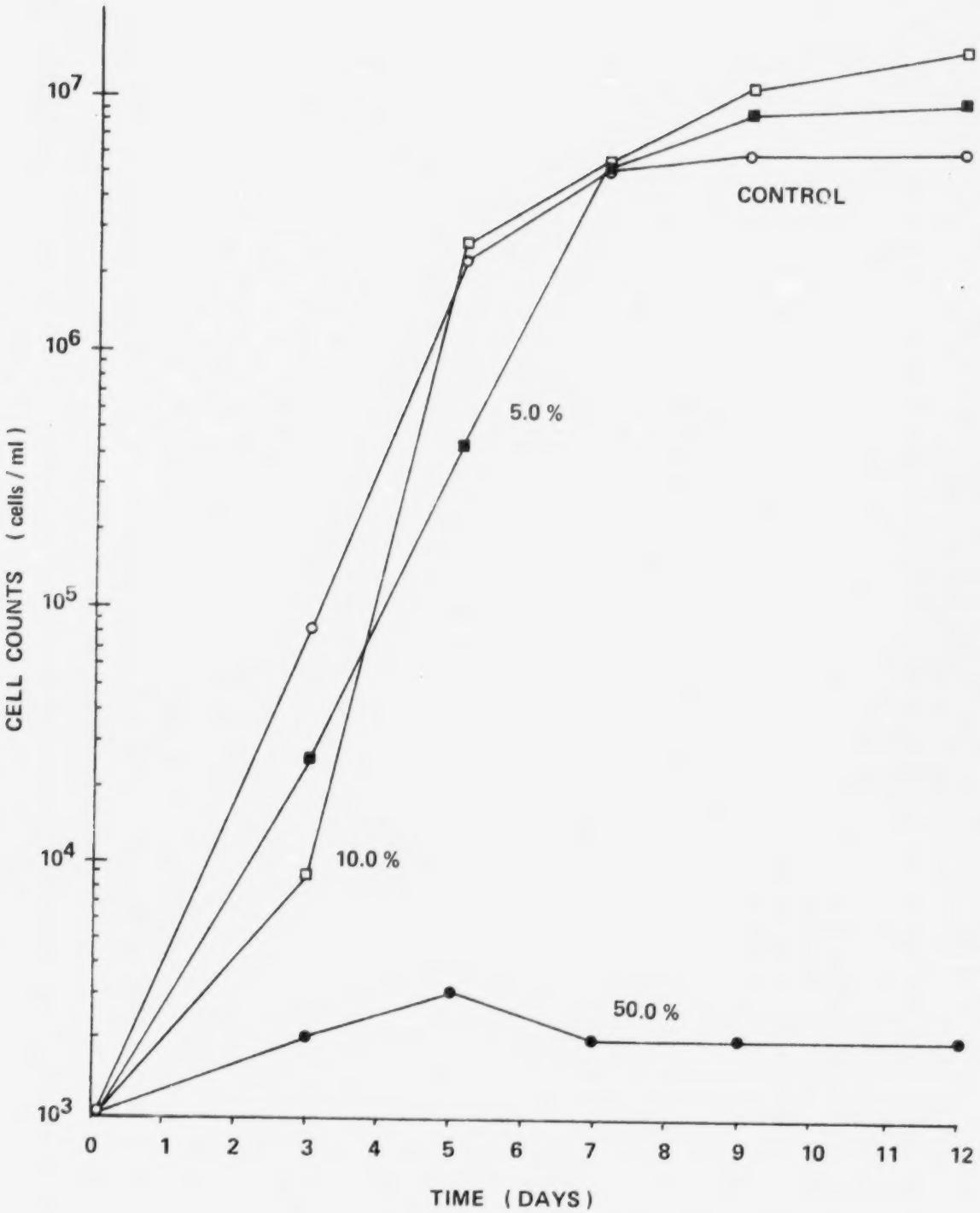


Figure 51. Growth of Selenastrum capricornutum exposed to various dilutions of 20-day reactor effluent.

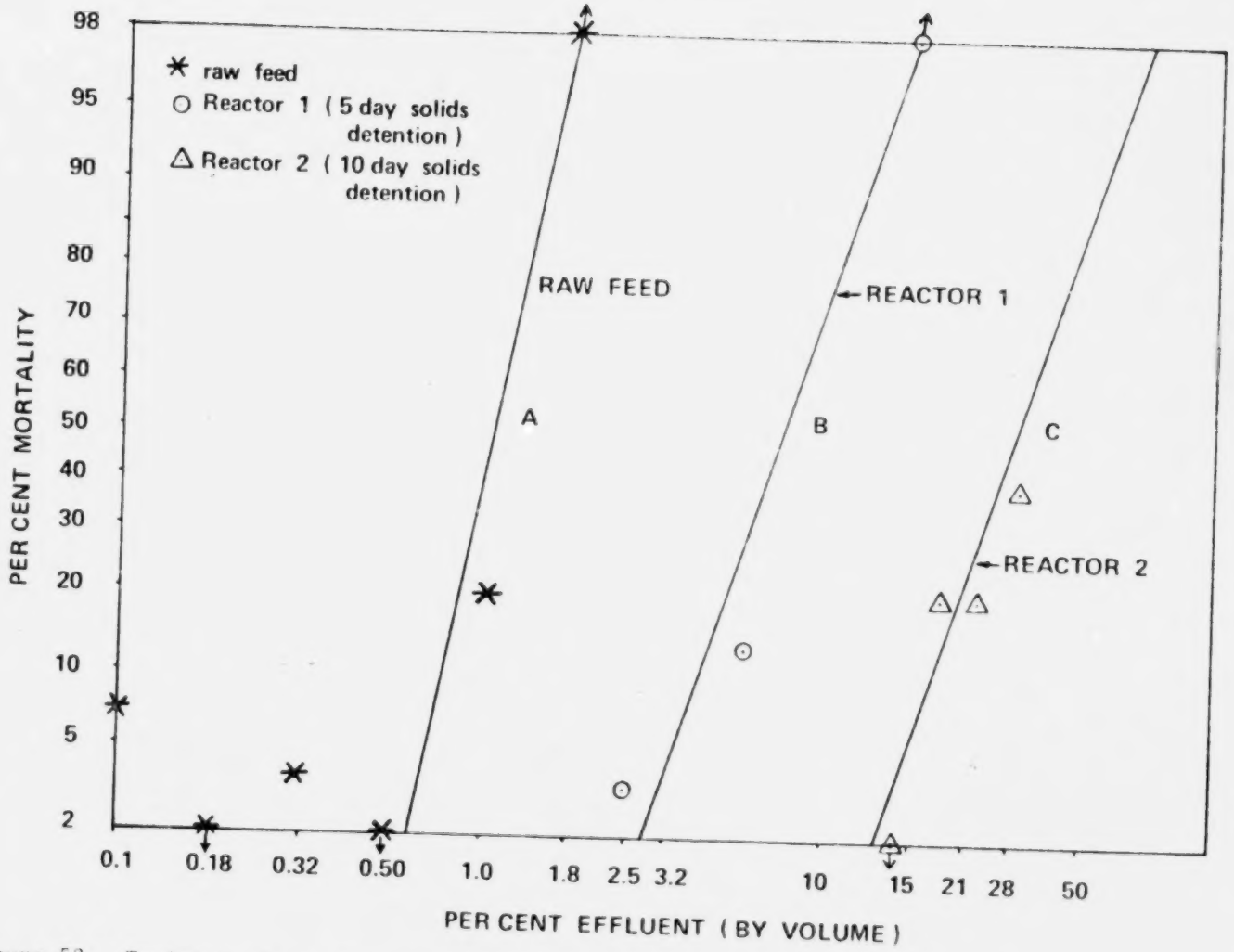


Figure 52. Toxicity of raw and biologically-treated synthetic wastewater to fathead minnows (Pimephales promelas).

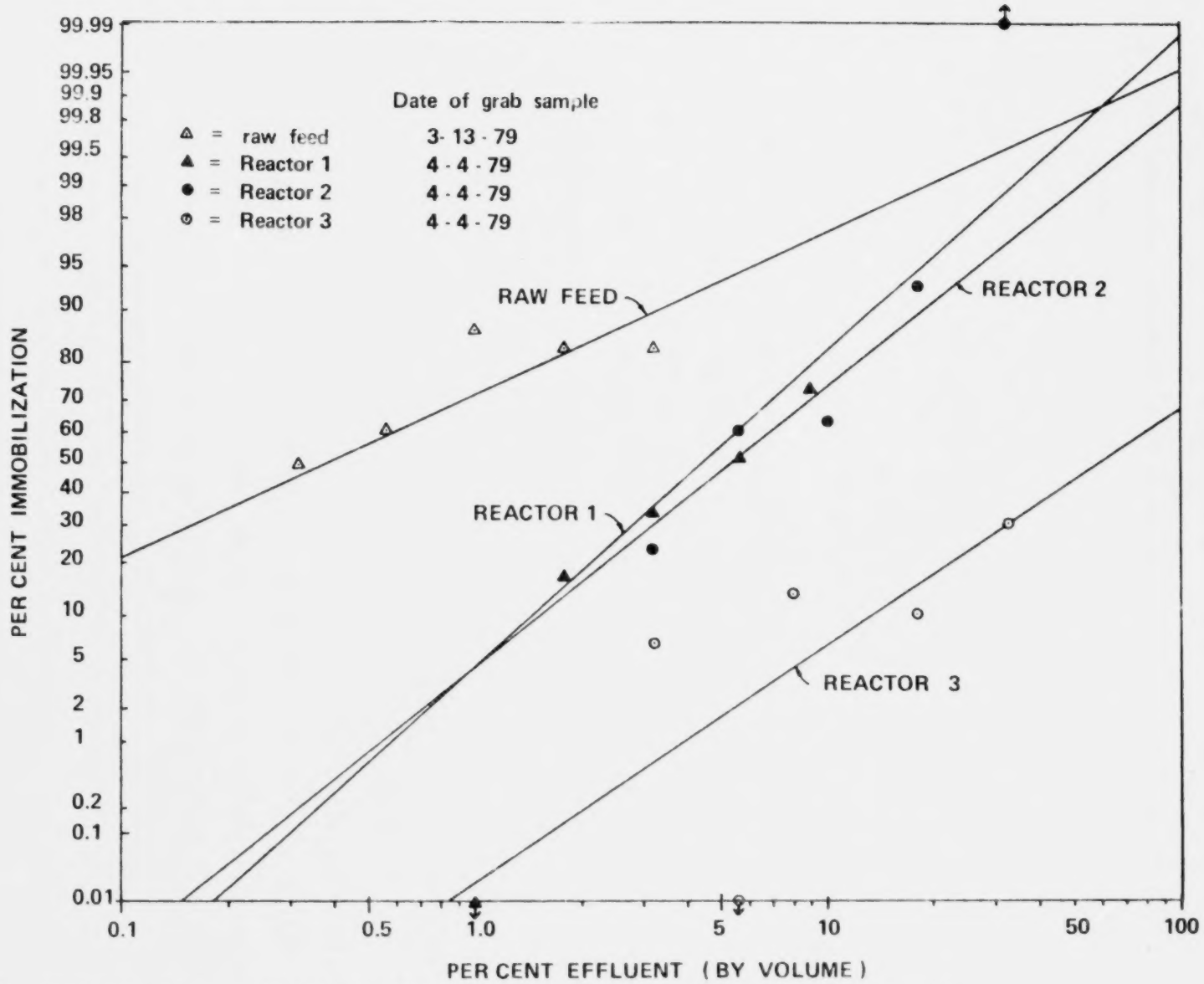


Figure 53. Toxicity of raw and biologically-treated synthetic wastewater to Daphnia pulex.

Although the sample collection and handling protocols discussed above appear to be appropriate for the fish and daphnia bioassays, the nature of the raw wastewater and the biologically treated effluents has hindered effective study of algal toxicity. In order to overcome the bacterial contamination problems associated with the algal assay procedure, an axenic (bacteria-free) culture of Selenastrum capricornutum has been isolated and will be used for future tests.

HEALTH EFFECTS BIOASSAY

A clonal toxicity assay, employing the Chinese hamster V79 cell line (13), was used to compare the relative acute toxicities of the effluents from the biological reactors and the raw synthetic wastewater. This assay measures the colony-forming ability of mammalian cells exposed to toxicants. The purpose of this test was to evaluate the effectiveness of biological treatment in alleviating potential human health effects associated with coal conversion wastewaters. While direct extrapolation of in vitro test results to in vivo conditions is difficult, cell culture methods are considered valuable for assessing the relative toxicities of environmental pollutants.

Effluent samples from the 10- and two 20-day reactors during the first series of biological treatability studies described in Section 4 were collected on September 17, 1978 and from the 5-day reactor on October 28, 1978. The samples were centrifuged and then filtered through a series of Nuclepore polycarbonate filters consisting of a 1.0 μm prefilter and a 0.2 μm ultimate filter. The filtrates were collected and aliquoted in small glass prescription bottles, which were then frozen and stored at $-80^{\circ}C$.

A sample of the raw synthetic wastewater which had been aged for two days was collected, treated, and stored in a similar manner. Individual aliquots of frozen reactor effluents and raw feed were thawed immediately prior to their use and the remainder of that aliquot was discarded at the end of the day.

A series of dilutions of each wastewater was made in distilled-deionized water. The addition of 2x or 4x nutrient medium to the dilution tubes maintained physiological conditions at final test concentrations ranging from 0.25 to 75% of the wastewater sample being tested. Two hundred cells were seeded per 60 mm tissue culture dish and allowed to incubate and attach for 3 hours in 3 ml of normal cell growth medium. Duplicate dishes were then treated with appropriate dilutions of a test wastewater. Each pair of dishes received a single concentration of the test materials. After an exposure period of 20 hours, growth medium containing the test materials was removed. The cells were washed once in a phosphate buffered saline solution and reincubated in 3 ml of fresh growth medium. Exposed single cells were allowed to grow into colonies and were then fixed and stained after 7 days. The number of colonies for each exposure condition was calculated as a percent of the number of colonies in untreated control plates, and expressed as the relative plating efficiency.

The results of the clonal toxicity assay are shown in Figure 54, where concentration-dependent survival curves have been plotted using the average of the data points from duplicate clonal toxicity experiments. The concentrations indicated represent dilutions of the samples being tested. Concentrations producing 50% lethality (LC_{50}'s) are shown in Table 20 along with the corresponding TOC concentrations of the wastewater sample

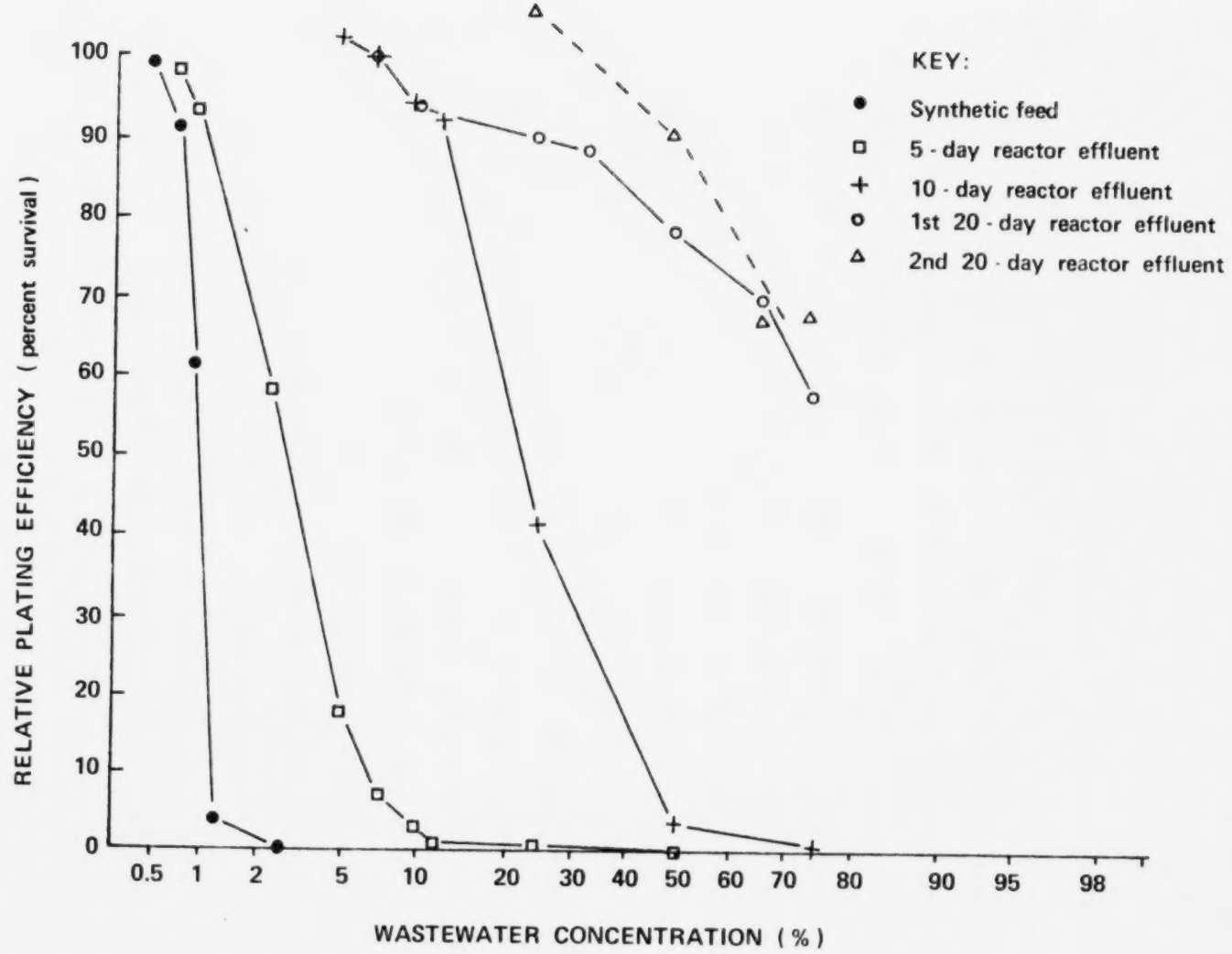


Figure 54. Results of 20-hour clonal toxicity assay using V-79 Chinese hamster cells.

TABLE 20. SUMMARY OF MAMMALIAN CYTOTOXICITY DATA

Sample	TOC mg/l	LC_{50} %
Raw wastewater	1600	1.0
5-day reactor effluent	200	3.0
10-day reactor effluent	80	23.5
First 20-day reactor effluent	45	80 *
Second 20-day reactor effluent	45	80 *

* The two 20-day reactors did not produce 50% lethality at the highest concentrations tested (75%). The LC_{50} values shown are extrapolated from the plots in Figure 54.

under examination. It is apparent from Figure 54 and Table 20 that V79 cytotoxicity decreases with increasing degree of wastewater treatment as measured by solids residence time.

It is interesting to note in Table 20 that while the 5-day reactor provided an 87.5% (8-fold) reduction in TOC compared to the raw wastewater, the LC_{50} was reduced only three-fold. This suggests that most of the easily-degradable TOC may not be very cytotoxic. On the other hand, the 95% (20-fold) TOC reduction produced by the 10-day reactor corresponded to a 23-fold reduction in cytotoxicity, while the 97% (33-fold) reduction in TOC produced by the 20-day reactors corresponded to an 80-fold reduction in cytotoxicity compared to the raw wastewater. This suggests that TOC removal, in itself, may not be a very useful indicator of toxicity reduction.

A similar series of acute cytotoxicity tests is currently being performed on the effluents from the second set of biodegradability studies described in Section 5. Additionally, the raw and treated wastewaters are being assayed for their mutagenicity potential, using the Ames test. The results of these further, more comprehensive health effects assays will be presented in the next report in this series.

SECTION 8

VOLATILITY AND AIR-STRIPPING OF ORGANICS DURING BIOLOGICAL TREATMENT

An experiment was performed to provide a preliminary estimate of the quantity and nature of the organics lost by air-stripping in the biological reactors. To simplify the data analysis, microorganisms were excluded by using fresh unseeded synthetic wastewater. The combined effect of biological activity and aeration on the production and potential loss of volatile organics will be addressed at a later date.

PROCEDURE

One of the reactors was filled with 26 liters of freshly-prepared 1/4-strength synthetic coal conversion wastewater. In addition, a BOD bottle was filled and stoppered to serve as a control. At no time was there any indication that air had been trapped in the BOD bottle. The control was stored alongside the reactor for the duration of the experiment (26 hours).

Initially, at time zero, a sample was taken from the bottom port of the reactor. Part of the sample was stored under refrigeration for future TOC measurement. The remainder was taken immediately to the analytical laboratory for HPLC analysis. Before the raw wastewater was one hour old, aeration was started at an air flow rate of 1 liter/min, corresponding to the air flow rate in the biological treatability studies. (The gas

transfer properties of the reactor are characterized by an overall mass transfer coefficient for oxygen, $k_L a$, of 1.8 hr^{-1}.) Samples were taken from the lower port at 1, 7.5, and 26 hours of aeration. These were handled in the same manner as the initial sample. In addition, the control was also sampled at 26 hours. Due to minor operational problems with the HPLC, analysis of all but the initial sample was delayed from several hours to two days. A final sample was collected after 97 hours for TOC analysis.

Total organic carbon was measured using the total carbon channel on a Beckman 915 Carbon Analyzer. A 10 ml aliquot was acidified with 2 drops of 50% HCl to decrease the pH below 4, and the sample was purged with nitrogen for 4 minutes, to strip out any inorganic carbon prior to injection into the TOC analyzer. The HPLC procedure was identical to that described in Section 7.

RESULTS AND DISCUSSION

The results of the TOC analyses are listed in Table 21. It is clear that no loss of volatile organic carbon resulted from aeration of the wastewater under conditions parallel to those in the biological treatability studies.

Table 22 lists the results of the HPLC analyses after various times of aeration. The reference HPLC chromatogram used for this purpose is shown in Figure 55. The chromatogram is similar to that presented in Section 7 for the raw wastewater, although some minor differences are apparent due to ageing of the column. It is recommended that the HPLC results be interpreted with some degree of caution since delays of a day or more between sample collection and analysis might have significantly affected

TABLE 21. CHANGE IN TOTAL ORGANIC CARBON CONCENTRATION RESULTING FROM AERATION (mg/l)

Time (hours)	TOC in Reactor	TOC in Control
0	1,083	
1	1,081	
26	1,068	1,076
97	1,110	

the integrity of the sample. It should be noted that the wastewater darkened almost immediately after aeration was initiated, whereas the sealed control remained relatively colorless. When the control was opened after 26 hours for sampling, it also began to darken due to exposure to air. Despite this change in the appearance of the sample, changes in the HPLC traces are relatively minor. There is some variability associated with peak #3 (resorcinol and catechol) which cannot be explained, and several of the minor peaks, primarily those of the relatively non-polar compounds, disappear after 26 hrs. In general, however, it can be concluded that there is little difference between the chemical composition of the aerated sample and that of the control.

CONCLUSIONS

The volatility of the synthetic wastewater constituents is sufficiently low that no significant loss in TOC can be attributed to aeration when only the constituents of the raw wastewater are considered and the wastewater is aerated under conditions likely to be encountered during biological treatment. If any volatile organics are produced during biological

TABLE 22. RESULTS OF HPLC ANALYSIS OF AERATED WASTEWATER

Peak No.*	Constituent	HPLC Sensitivity#	Concentration in Raw Wastewater (mg/l)	PEAK HEIGHT+ Aerated Samples 0 hrs	1 hr**	1 hr**	7.5 hrs	26 hrs	Control 26 hrs
1	Benzoic Acid	8.1	25	3.0		3.1	0.2	0	0
3	Resorcinol	4.4	250	77.0		69.2***	114	91.3	94.0
	Catechol	28	250						
4	Analine	1.6	5	0		0	0	0	0
5	Phenol	6.4	500	71.6	70.0	69.2	69.2	67.2	68.9
	5-Methylresorcinol	42	12.5						
6	4-Methylcatechol	7.4	25	2.7	2.6	2.9	2.1	1.9	2.4
7	o-Cresol	3.0	100						
	p-Cresol	20	62.5	43.8	42.5	42.4	42.8	42.0	42.8
	2-Indanol	1.0	12.5						
8	Acetophenone	6.0	12.5	3.4	3.4	3.3	4.0	4.1	3.8
9	3,4-Xylenol	2.2	62.5						
	Pyridine	3.4	30	43.0	42.2	42.0	38.0	32.0	38.0
	Quinoline	1.3	2.5						
10	Indole	0.87	12.5	18.5	22.0	19.5	17.5	12.5	15.5
	2,3-Xylenol	6	62.5						
11	3,5-Xylenol	5	10	0	0	0	0	0	0
12	2-Naphthol	1.4	12.5	16.4	14.2	14.8	5.0	2.0	8.8
	2-Methylquinoline	1.2	10						
13	3-Ethylphenol	3.1	7.5	4.6	3.3	3.2	4.0	4.0	5.2
14	1-Naphthol	0.58	5	4.5	4.2	4.0	2.0	0.6	0
	2,3,5-Trimethylphenol	21	12.5						
15	Indene	8.3	12.5	1.1	0.9	0.8	1.2	0	1.2
16	Naphthalene	0.9	1.25	1.4	1.1	1.1	0.9	0	1.4

+ 10 microliter sample, UV-absorbance at 280 nm, values in 0.0005 absorbance units.

* see Figure 55

HPLC sensitivity in mg/l per 0.0005 absorbance units.

** A duplicate of the 1 hour sample was made as peaks 1, 3 and 4 were inadvertently lost on the first injection.

*** Peak #3 appeared with a shoulder here. This could indicate that two closely overlapping peaks created the erratic results.

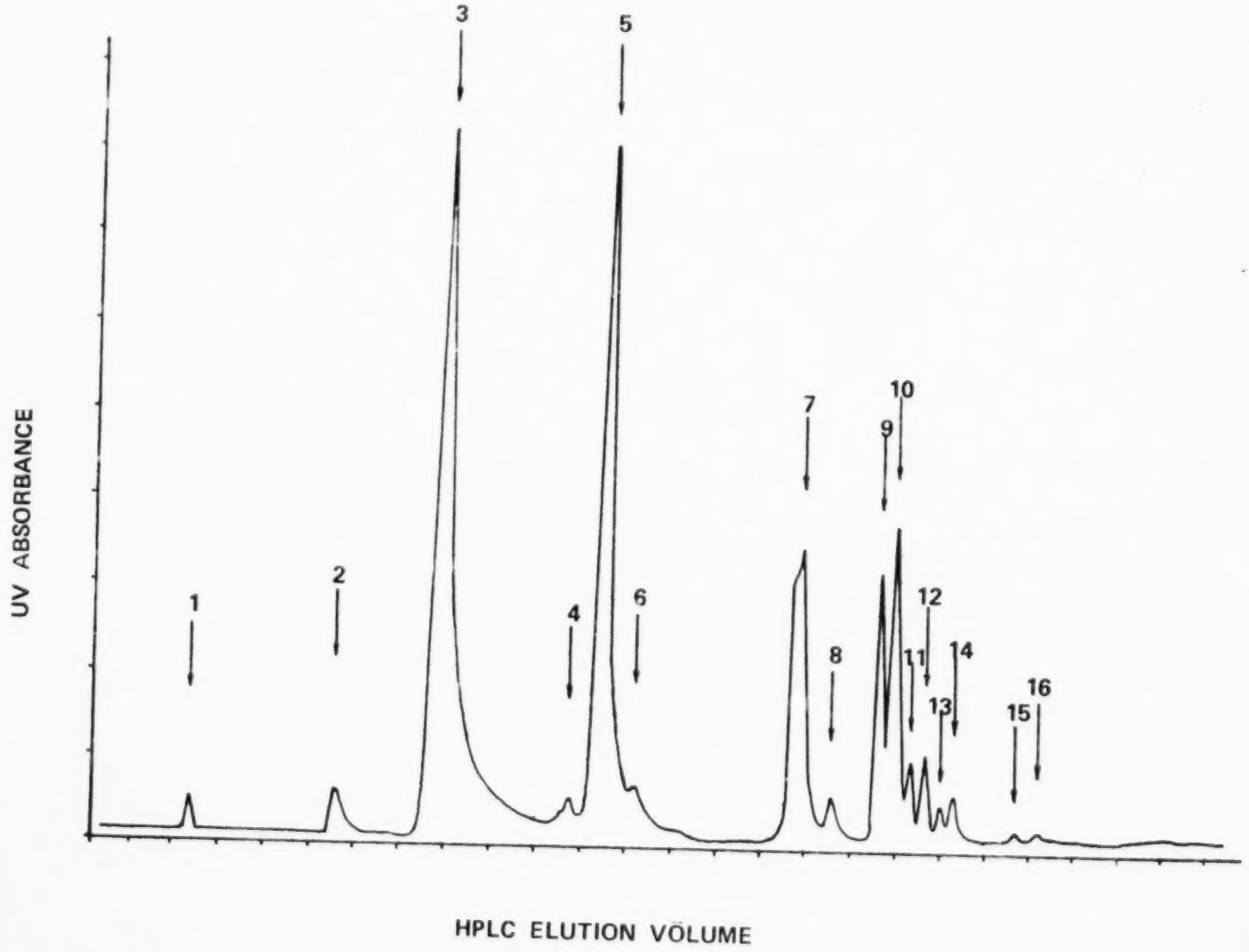


Figure 55. HPLC chromatographic profile of synthetic wastewater for stripping evaluation. (Numbers correspond to compounds in Table 22.)

treatment, however, it is conceivable that they may be stripped during aeration. However, this remains to be proven under normal conditions of aeration.

SECTION 9

BIODEGRADABILITY OF COAL CONVERSION WASTEWATER CONSTITUENTS

The reactors used to evaluate the biological treatability of coal conversion wastewaters are essentially microbial oxidative degradation systems. A variety of microorganisms utilizing many different pathways are involved in the biodegradation of the aromatic and aliphatic molecules in the raw wastewater being fed to the reactors. Interpretation of the results of reactor operation can be greatly aided by knowledge of the biodegradation pathways used by the microorganisms for the various individual components of the wastewater. Knowledge of biodegradation rates and pathways can also provide insight as to metabolic products that might accumulate and be present in the reactor effluent and to identify whether or not certain substrates might act as metabolic inhibitors.

An initial screening analysis with respect to the biodegradability of coal conversion wastewater constituents using biochemical oxygen demand (BOD) techniques and an unacclimatized microbial seed was described in an earlier report (1). That analysis revealed preliminary information about the potential biodegradability of fifty-one constituents of coal conversion wastewaters. In this section, the results of a more complete biodegradability study, utilizing standard manometric techniques (14) and an acclimated seed from the biotreatability reactors, are described.

PROCEDURE

Two hundred ml of mixed liquor from one of the 20-day reactors was centrifuged for about 15 minutes. The supernatant was decanted and the solids were resuspended in 200 ml of 0.01M phosphate buffer. The suspension of seed organisms was aerated for 15 to 30 minutes to allow metabolism of any remaining wastewater constituents. A subsample of approximately 50 ml was removed from the flask and autoclaved, to be used ultimately as a sterile control.

Volume-calibrated manometers and Warburg flasks were used. Eleven ml of aerated seed was pipetted into the reaction compartment of each Warburg flask. Eleven ml of autoclaved sterilized seed was also added to one flask of each compound as a control. Three ml of 10% KOH and one piece of accordion-folded filter paper was added to the center well of each flask.

All substrate solutions were prepared in 0.01M phosphate buffer. This concentration allowed 0.6 mg in the 1.0 ml added to the 11 ml seed, giving a final concentration of 50 mg/l in each flask. Each substrate was run in duplicate or triplicate and each had a sterile seed control. For each experiment, two flasks received no chemical addition, receiving instead 1.0 ml of phosphate buffer. These flasks comprised the controls for endogenous O_2 uptake. A thermobarometer containing approximately 15 ml of buffer was included in each run to correct for changes in ambient temperature and pressure. The manometric apparatus was assembled and each flask was placed so that it would be immersed in the 30°C water bath. Flasks were allowed to equilibrate for 20 minutes with the stopcock open. After equilibration, at time zero, the stopcock was closed, fluid in the manometer was adjusted to the 250 mm level in the closed arm and the reading was recorded from the

open arm. This procedure was repeated every ten minutes, thereby keeping volume constant. After 10 minutes of incubation to allow pressures to stabilize, the chemical contents (substrate) in each side arm were carefully tipped into the reaction flask. The duration of the O_2 uptake measurements lasted from 3 to 5 hours. At the end of each experiment, subsamples of the reaction flasks were centrifuged to remove solids. These samples were frozen and analyzed within several days by gas chromatography to determine the concentration of substrate remaining. Initial samples of each substrate solution were also analyzed.

CALCULATIONS AND RESULTS

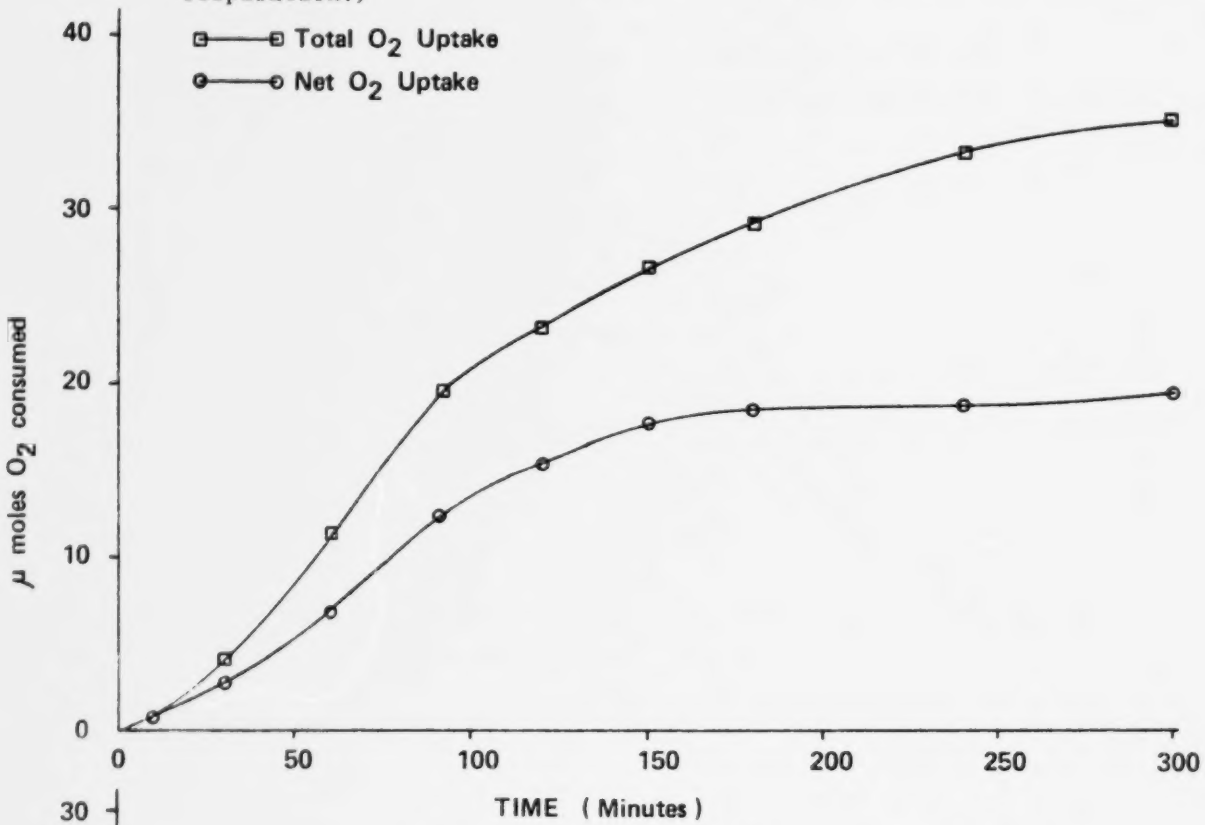
Changes in the fluid level of the thermobarometer (TB) were substracted from net changes in each manometer to obtain TB-corrected net changes. Cumulative sums of oxygen uptake were computed and multiplied by the appropriate flask constant to obtain the amount of gas exchanged in microliters, for the appropriate time intervals. Flask constants were previously calculated from known parameters of temperature, pressure, fluid volume, gas volume, and solubility (14). Net oxygen uptake was determined by subtracting endogenous oxygen uptake from the gross uptake measured by the respirometer. Replicates for each compound were averaged and converted to µmoles O_2 uptake. From the analytical data, the amount of substrate originally added to each flask was determined and converted to µmoles. This value was multiplied by a factor f, determined from the balanced equation:

$$\text{substrate} + f\ O_2 \rightarrow CO_2 + H_2O \qquad (9)$$

to obtain the umoles of O_2 required for complete oxidation of the known amount of substrate. The percent of the theoretical oxygen demand (THOD) was computed by dividing the O_2 utilized during the experiment by the O_2 required for complete oxidation and multiplying by 100. On the graph for each substrate (see Figures 56 through 64), gross umoles O_2 uptake due to substrate utilization, and net uptake (endogenous uptake subtracted from gross uptake) were plotted.

In an effort to obtain information on how readily the substrates were degraded, an initial rate of oxygen uptake was determined for each compound. From the graphs, the total umoles of O_2 utilized per hour during the linear phase of oxygen uptake was divided by grams of mixed liquor suspended solids in 11 ml of reactor contents to obtain these initial rates shown in Table 23. Data on mixed liquor suspended solids (MLSS) was obtained from reactor operating data. These values cannot be directly compared as they depend to an extent on the initial amount of substrate added which varied somewhat among different compounds. Note that Table 23 also includes sterile substrate concentrations or the amount of substrate remaining in the reaction flask which contained autoclaved seed. Theoretically, no biological oxidation should have occurred. Therefore any decrease from S initial to S sterile may be due to chemical oxidation or adsorption of the chemical to solids. In a few cases, S sterile is larger than S initial. The most likely explanation involves the difficulty

Figure 56. Rate of oxygen utilization resulting from microbial degradation of phenol. (Net oxygen uptake equals total oxygen uptake less endogenous respiration.)



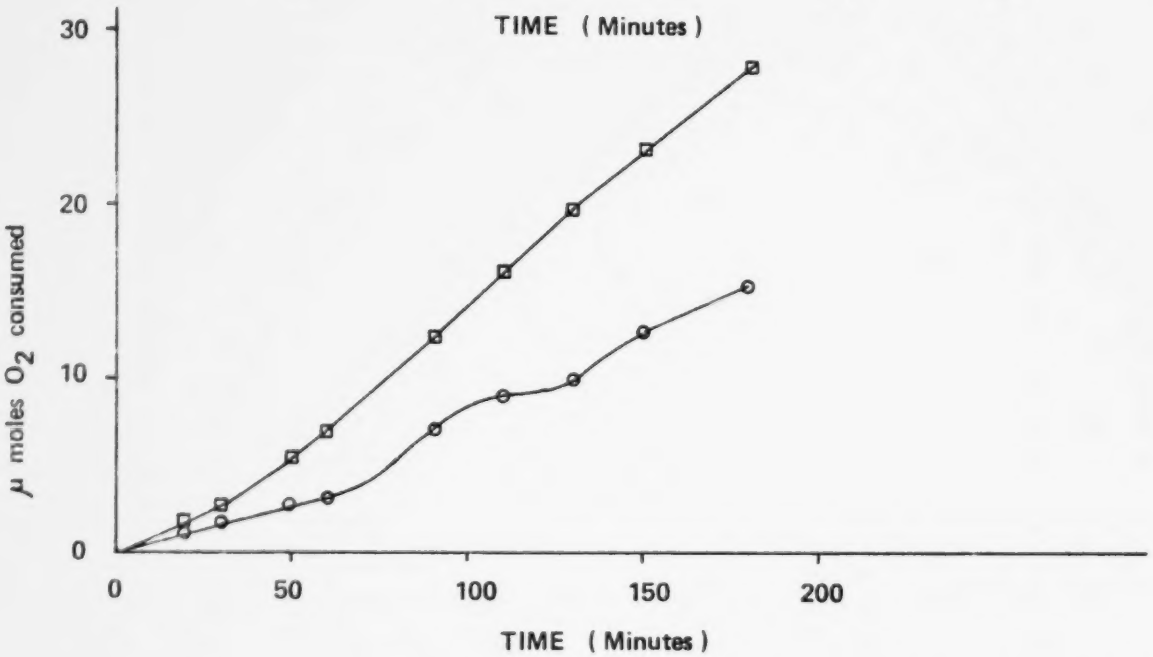


Figure 57. Rate of oxygen utilization resulting from microbial degradation of p-cresol.

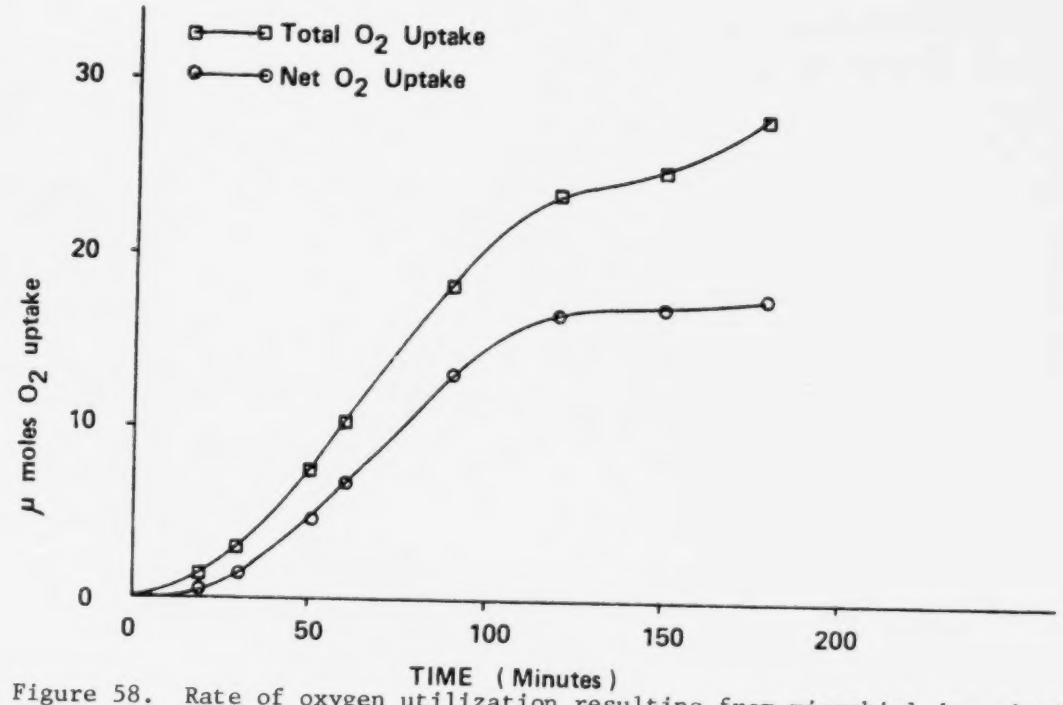


Figure 58. Rate of oxygen utilization resulting from microbial degradation of o-cresol.

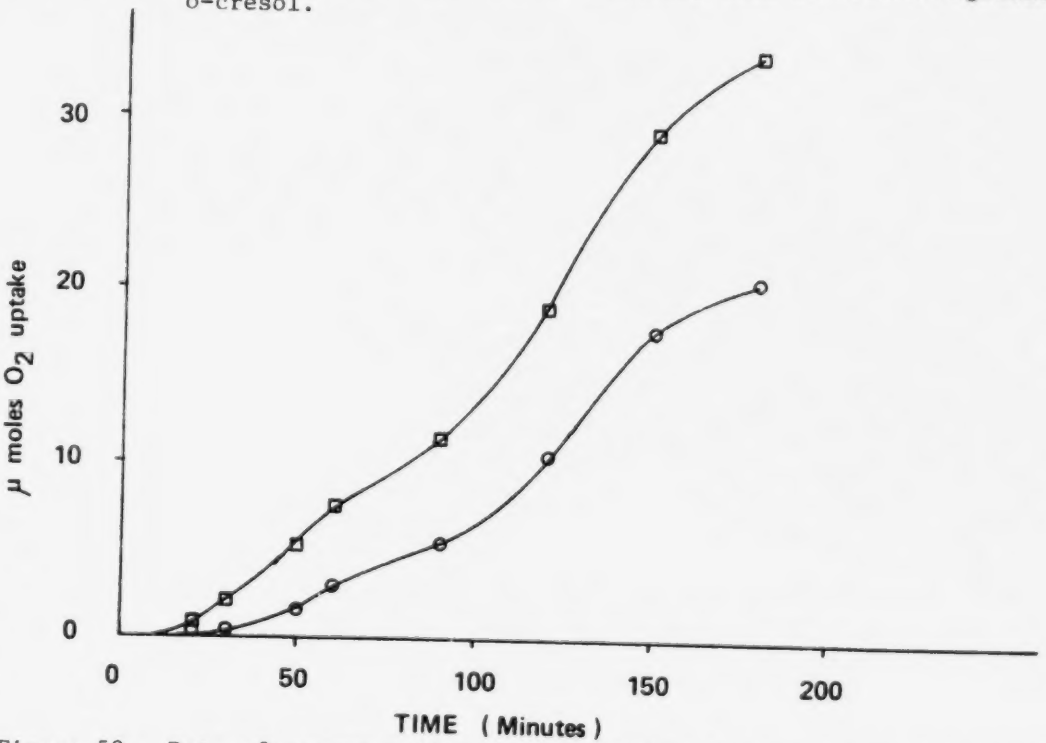


Figure 59. Rate of oxygen utilization resulting from microbial degradation of m-cresol.

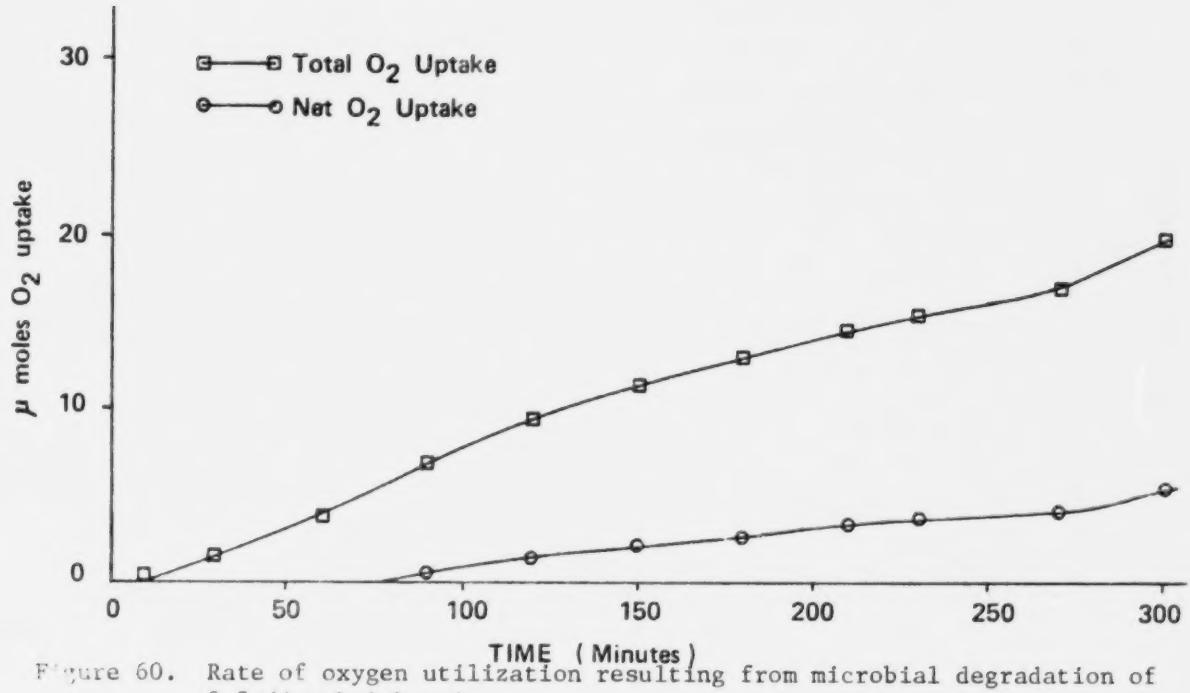


Figure 60. Rate of oxygen utilization resulting from microbial degradation of 2,5-dimethylphenol.

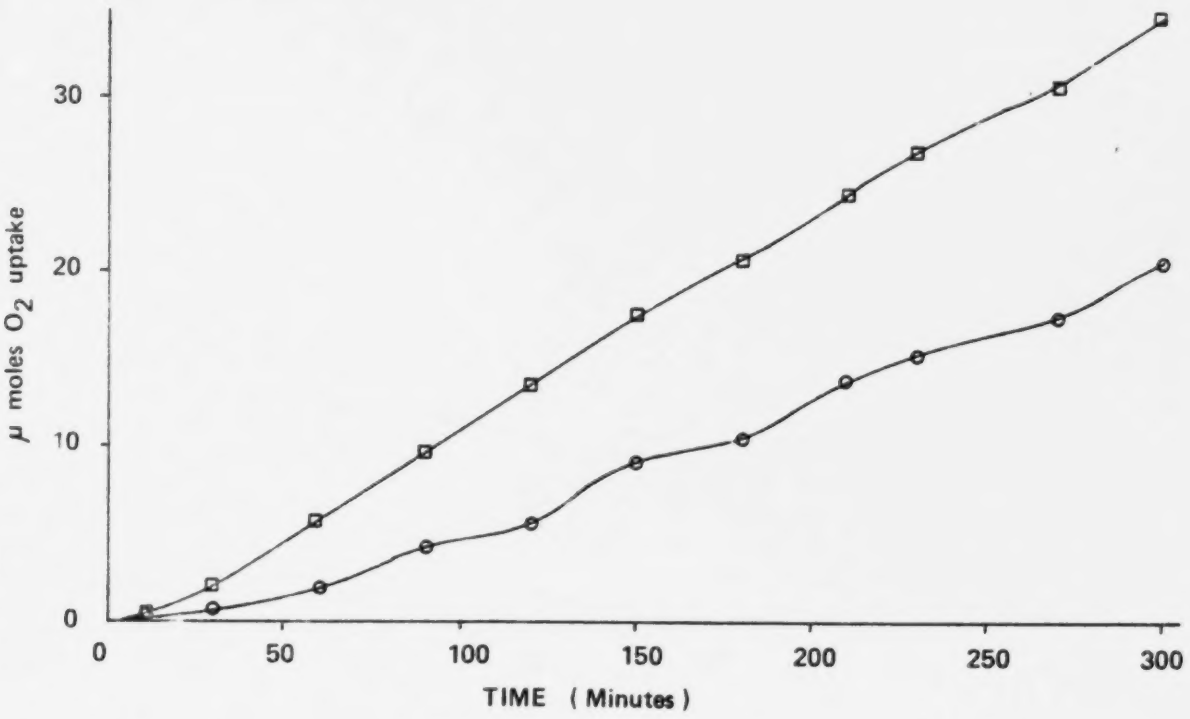
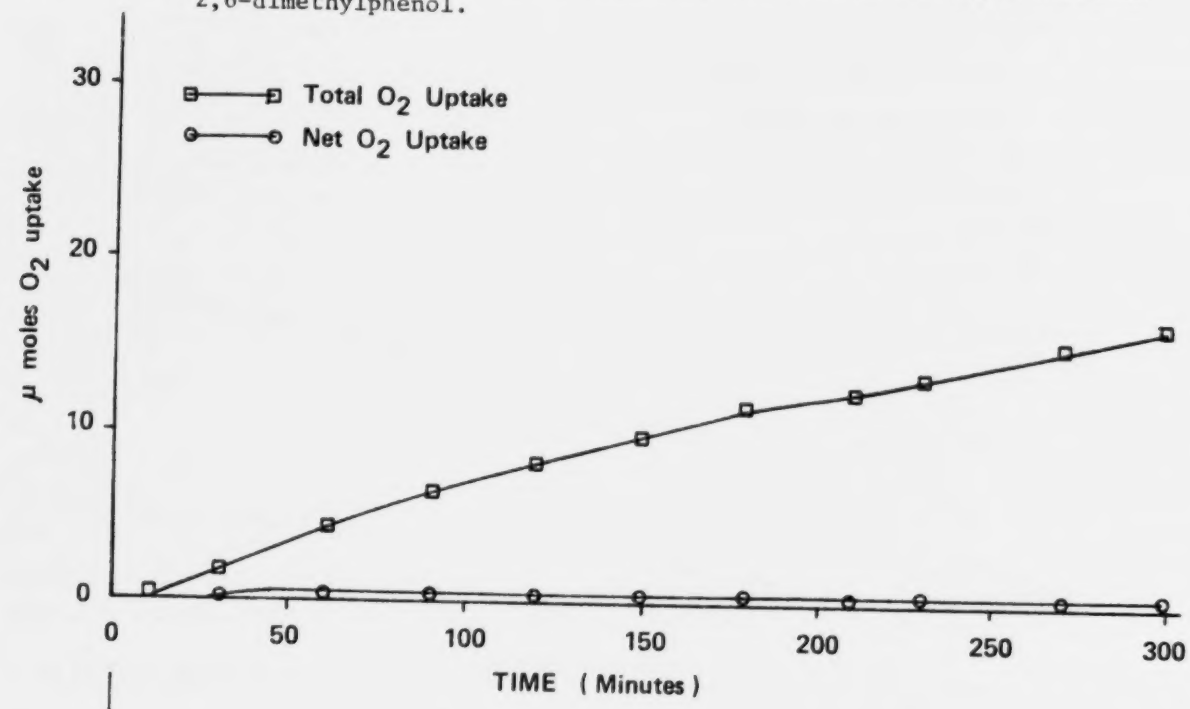


Figure 61. Rate of oxygen utilization resulting from microbial degradation of 2,3-dimethylphenol.

Figure 62. Rate of oxygen utilization resulting from microbial degradation of 2,6-dimethylphenol.



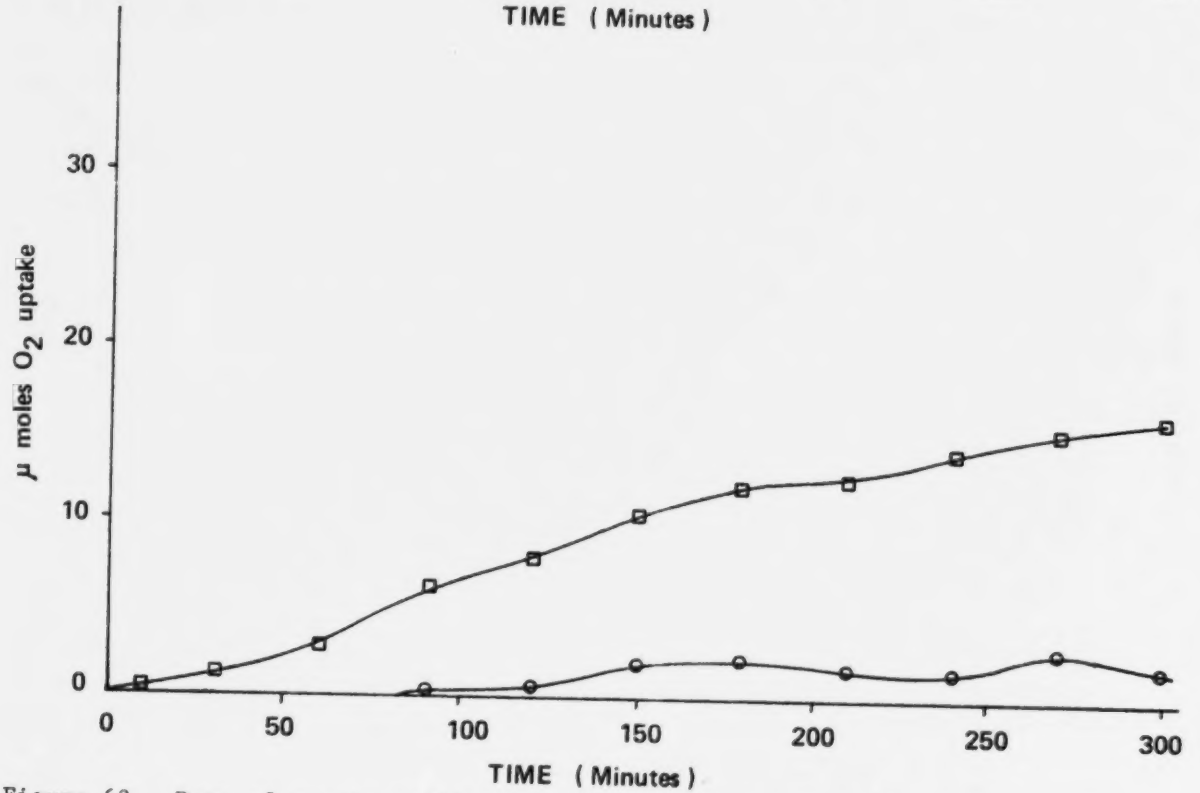


Figure 63. Rate of oxygen utilization resulting from microbial degradation of 3,5-dimethylphenol.

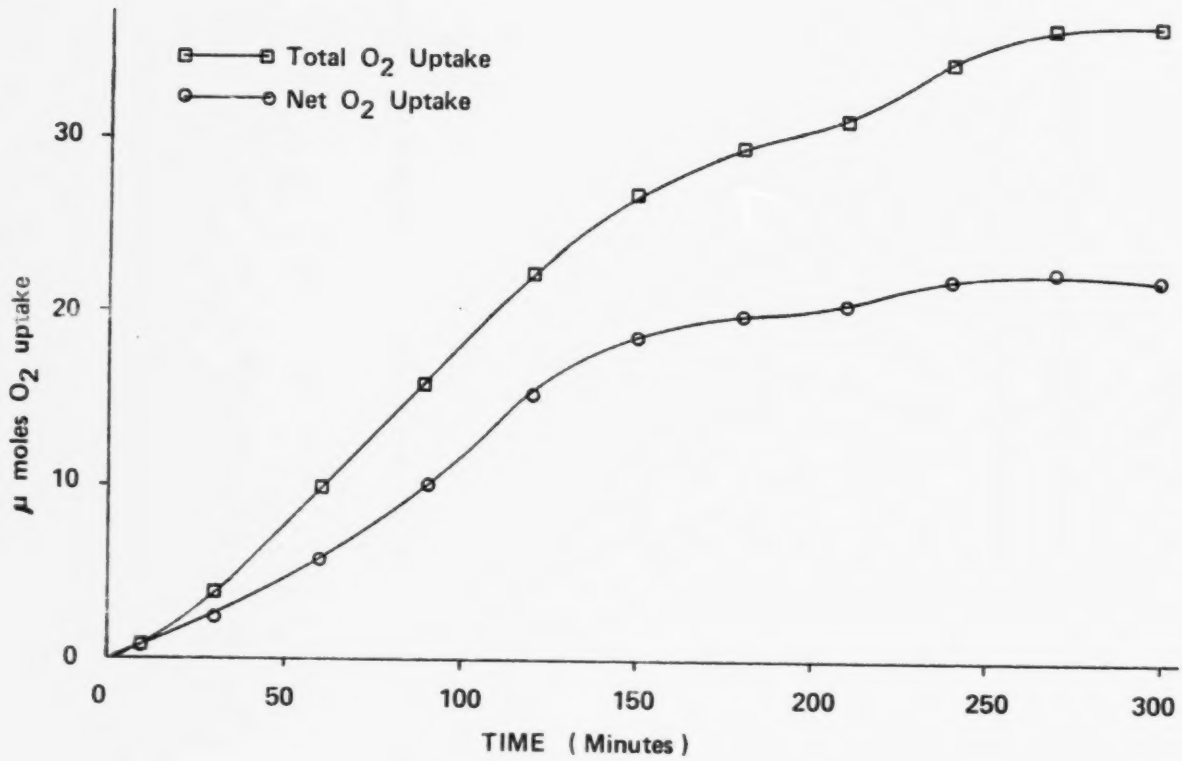


Figure 64. Rate of oxygen utilization resulting from microbial degradation of 3,4-dimethylphenol.

TABLE 23. BIODEGRADATION OF SELECTED COAL GASIFICATION WASTEWATER COMPONENTS

Substrate	Date (1979)	MLSS (mg/l)	Si (mg/l)	S_f (mg/l)	$S_{sterile}$ (mg/l)	THOD (mg/l)	THOD (umoles)	% THOD	Initial Net Rate of O_2 Uptake (umole O_2/hr-g MLSS)
Phenol	3/28	617	41.7	0	27	99.2	37.2	52.4	1.238
p-Cresol	3/7	766	29.1	1.0	24	73.3	27.5	56.4	0.570
o-Cresol	2/23	794	38.2	0.5	46	96.3	36.1	48.5	0.893
m-Cresol	3/7	766	50	3.3	38	126.0	47.3	43.3	0.807
2,6-Dimethylphenol	3/28	617	50	25.5	29	131.0	49.1	1.0	0.015
2,5-Dimethylphenol	3/17	615*	50	29.7	50	131.0	49.1	11.2	0.163
2,3-Dimethylphenol	3/17	615*	50	1	40	131.0	49.1	42.8	0.606
3,5-Dimethylphenol	4/6	643	46.7	21.7	33	122.4	45.9	5.4	0.057
3,4-Dimethylphenol	4/27	701	50	0.7	64	131.0	49.1	45.8	0.973

*Estimated

of tipping the entire side arm contents into the reaction flask at the beginning of the experiment. Some variability results.

DISCUSSION

In this phase of the investigation, essentially three groups of molecules were examined. Phenol constitutes one group, the cresols, with three different isomers, the second group, and five isomers of the xylenols the third. The overall pattern of metabolism observed was consistent with expectations based upon laboratory studies published in the literature (15). Phenol was degraded extensively and at the highest rate, the cresols as a group being less biodegradable than phenol, but more degradable than the xylenols. This follows the general pattern that has been observed in laboratory studies of rates of biodegradation that total amounts of biodegradation are less when increasing numbers of substituents are added to aromatic ring systems.

The xylenols were metabolized to a lesser extent than either of the other groups, but still showed significant amounts being degraded over a five hour period. The pattern of metabolism for the different isomers of xylenol reflects the usefulness of laboratory studies with pure cultures in predicting the biodegradability of components in mixed systems with mixed cultures. The two isomers of xylenol showing the largest amount of metabolism were the 2,3 and 3,4 isomers. In both cases, the two methyl groups are ortho to one another. The three other isomers in which the methyl groups were either meta or para to one another showed lesser amounts of metabolism. This is most likely the result of steric interference with

the second hydroxylation of the ring, which is necessary before ring opening and further metabolisn can proceed.

Further studies of the microbiological degradation of specific individual components in the coal gasification wastewaters are currently underway using seed organisms from the reactors. These studies will concentrate initially on the degradation of other components suspected to be present in real coal gasification wastes. Subsequent studies will focus on the degradation of mixtures of these compounds.

SECTION 10

MIXED LIQUOR RESPIRATION STUDIES

Early in the project it was recognized that there were several compounds in coal conversion wastewaters with potential for exerting toxic effects on biological treatment systems. This raised significant questions about practical treatability, and the wastewater concentrations at which treatment systems might perform satisfactorily, as well as other environmental factors influencing their operation. Accordingly, it was deemed advisable to initiate screening studies suitable for short-term evaluation of wastewaters to determine:

1. biodegradability of wastewater constituents under conditions as close as possible to those in operating reactors, and
2. toxicity of the constituents to mixed cultures present in the reactors, including approximate concentration levels at which such toxic effects might occur.

Several techniques have been employed by past investigators (16-22) to evaluate effects of wastewater constituents. The procedure selected for use here was developed several years ago in the laboratories of the University of North Carolina and subsequently has been applied by many investigators under widely varying conditions to assess acute effects of chemicals and wastewaters on wastewater treatment systems (16, 23, 24). The rather simple experimental setup is shown in Figure 65. Details of the

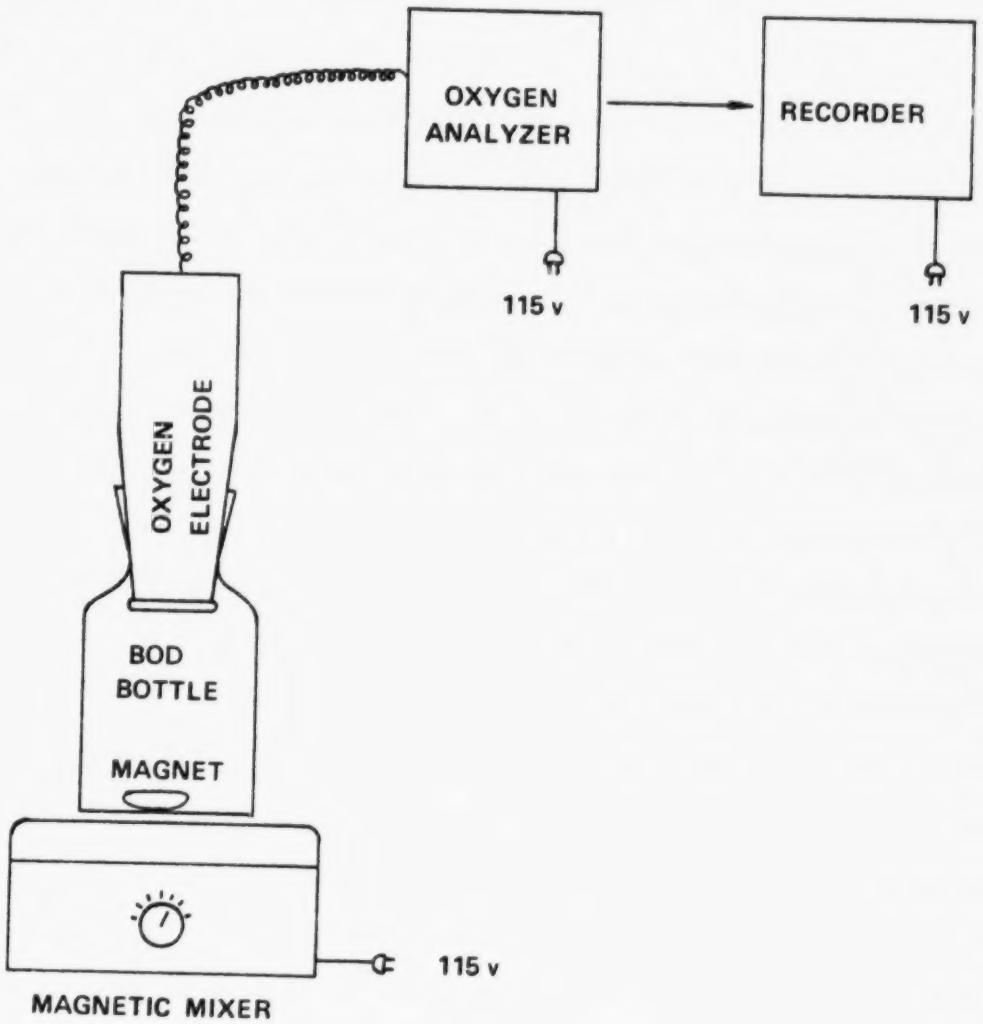


Figure 65. Experimental set-up for mixed liquor respiration studies.

concepts and techniques have been described earlier (16), and may be summarized here as follows:

(a) Samples of mixed liquor from a biological reactor are aerated for several hours to ensure that the mixed culture approaches "endogenous" respiration.

(b) A BOD bottle is filled with the mixed liquor, agitated continuously to maintain homogeneous conditions, and the endogenous respiration rate is measured using a dissolved oxygen (DO) electrode.

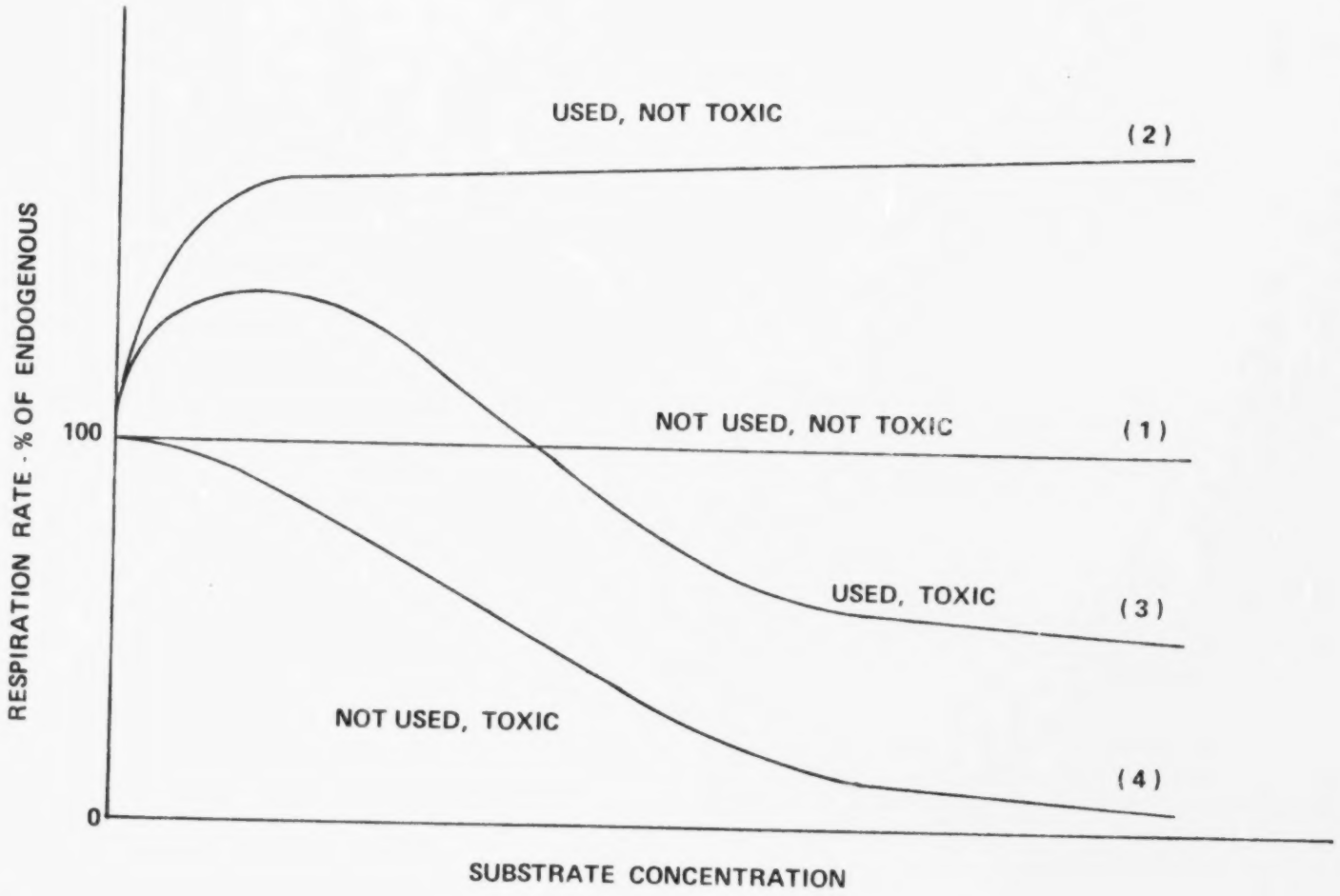
(c) A known quantity of the wastewater or chemical being evaluated is added to the sample and the new oxygen uptake rate is determined. Increases in oxygen uptake rates indicate higher levels of metabolic activity, usually because of biological utilization of the added constituents. Decreases in respiration rates indicate inhibitory effects on some of the organisms by toxic constituents.

(d) This procedure is repeated with several aliquots of mixed liquor at different concentrations of wastewater or constituents, usually extending from concentrations well below those in the actual wastewater to substantially higher levels.

Figure 66 shows the types of curves which have been observed commonly with this technique, with respiration rates plotted as a function of added substrate concentration. Curve 1 illustrates the situation which might be observed when a chemical which is neither used by the culture nor inhibitory to it is added to the respirometer. As the substrate concentration is increased, the respiration rate does not depart significantly from that of the endogenous sample, indicating no significant effect on metabolism of organics in the system. Curves 2, 3, and 4 illustrate the types of relationships which have been observed for chemicals having various characteristics with respect to utilization by the organisms and toxic impact on their metabolic activities.

Figure 67 summarizes data obtained in this fashion for several constituents of the synthetic wastewater. The level of each constituent is

Figure 66. Common types of oxygen utilization curves.



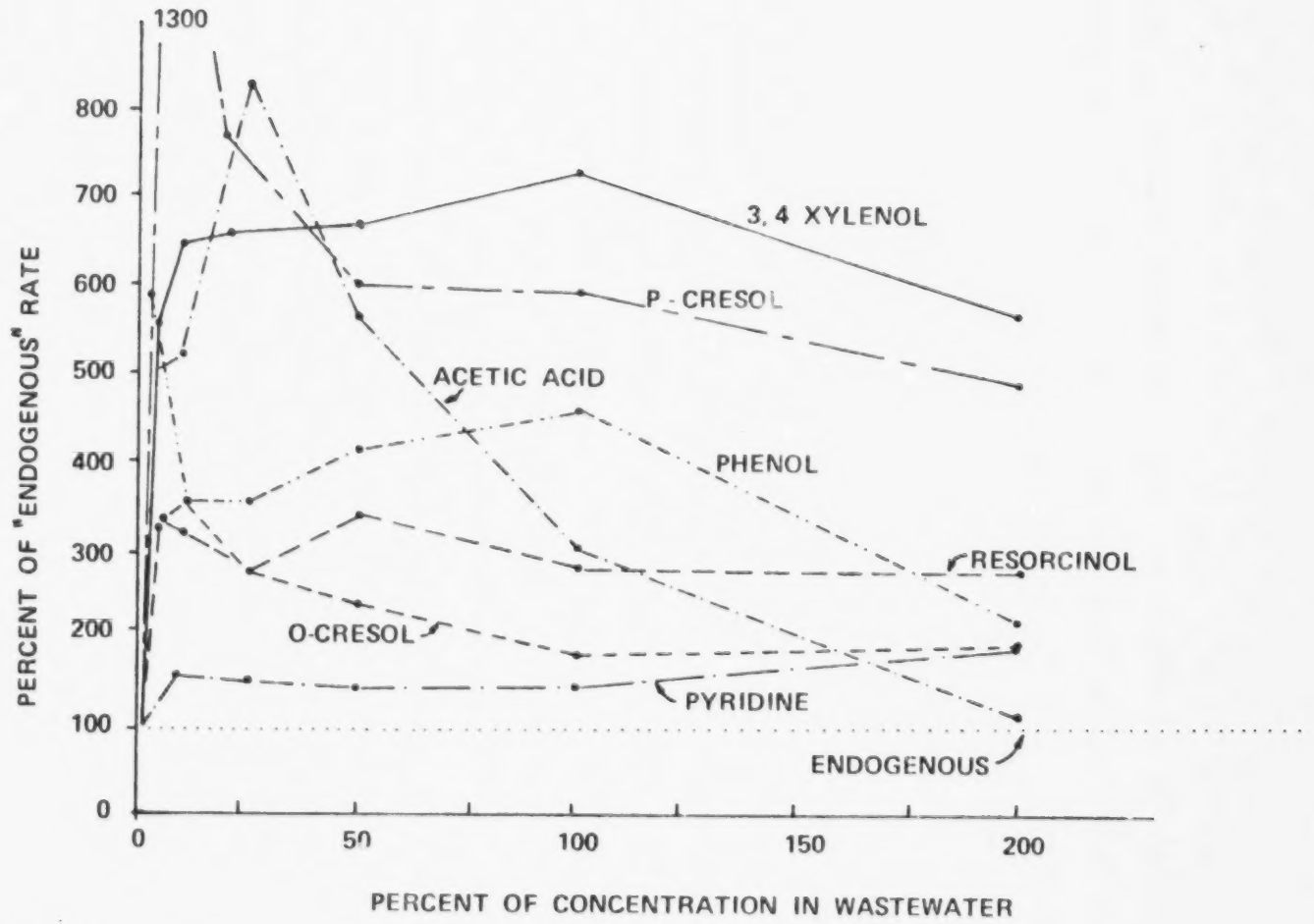


Figure 67. Respiration curves using original oxygen utilization procedure.

expressed as a percentage of its concentration in the full-strength synthetic wastewater. Respiration rates are expressed as percent of the "endogenous" rate for the sample of mixed liquor to which the constituent was added.

Although the techniques used in these preliminary studies have proven valuable in many earlier applications, some of the results raised concern because effects of certain chemicals were not consistent with data reported by other investigators (16, 23). For example, Figure 67 shows that the respiration rate for phenol increased sharply with increased phenol addition at low concentrations and continued to rise at phenol concentrations up to 100% of that in the synthetic wastewater (2,000 mg/l). Even at 200% (4,000 mg/l), phenol did not inhibit biological activity enough to depress respiration below the original endogenous rate. This is inconsistent with observations by previous investigators who have reported inhibitory action by phenol at concentrations at or above about 500 mg/l (25). Another problem encountered was development of respiration rate curves as a function of substrate concentration which were inconsistent with past experiences in these types of analyses. Difficulty also was frequently encountered with reproducibility of results, in general.

A series of tests was executed in which several aliquots of endogenous mixed liquor were dosed with different concentrations of phenol and samples removed from each periodically to determine respiration rate. At low concentrations, the respiration rate for sludge which had been aged for about 24 hours before adding phenol increased with time, as shown in Figure 68. Table 24 shows that "fresh" samples of mixed liquor were capable of using phenol at substantially higher rates than the aerated sludge

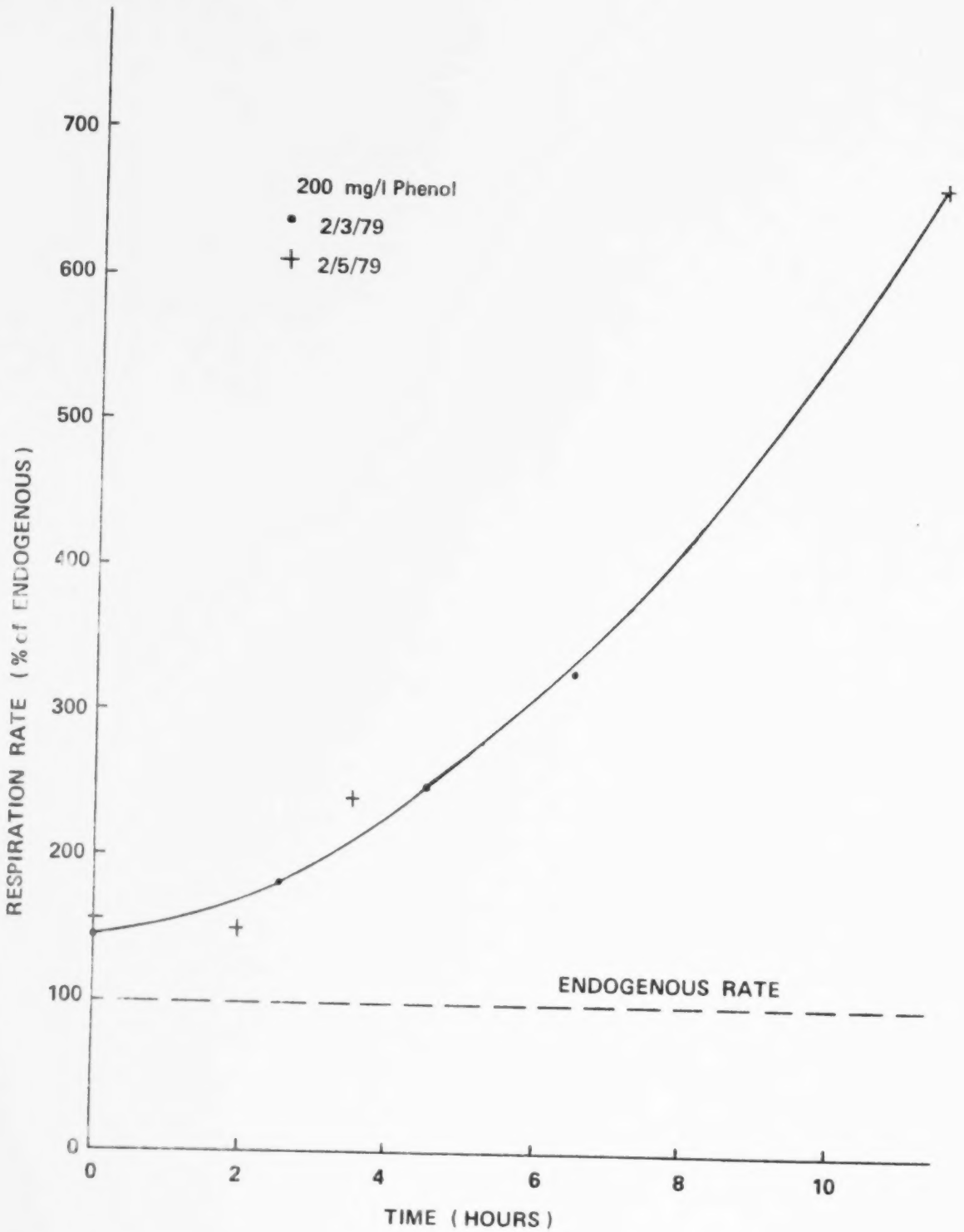


Figure 68. Respiration rate of aerated sludge with 200 mg/1 phenol.

TABLE 24. EFFECT OF AERATION ON "ACTIVITY" OF SLUDGE

Sludge Sample	RESPIRATION RATES, ug O_2/l-min-g MLSS	
	Initial Rate	With 500 mg/l Phenols
Aerated 24 hours (2/3/79)	0.21	0.31
Aerated 24 hours (2/5/79)	0.24	0.37
Aerated 24 hours (2/28/79	0.17	0.25
"Fresh" sludge from Reactor (4/11/79)	0.25	0.54

immediately after phenol addition. These data suggest that organisms in the system might have lost some of their ability to utilize the chemical during the period of aeration. At elevated phenol concentrations, the respiration rates were observed to decrease steadily over a period of several hours, as illustrated in Figure 69, showing progressive inhibition of biological activity by the phenol, at an initial phenol concentration of 2000 mg/l.

All of the difficulties led to the conclusion that experimental procedures should be modified to yield results which are more amenable to accurate interpretation, and to allow better reproducibility. The new procedure consists of removing mixed liquor from the reactor and determining its "initial" respiration rate immediately. Several checks indicated that in reactors producing high levels of TOC removal, respiration rates do not drop significantly over a period of 2 to 3 hours, as illustrated by Figure 70. This indicates that relatively little excess food exists in these reactors and that the organisms are in a metabolic state approaching endogenous respiration. Whether truly endogenous or not, the respiration rates are stable for extended periods, and can be demonstrated to be reproducible. After determining the "initial" respiration rate, aliquots of mixed liquor are dosed at different levels with the chemical in question and oxygen uptake rates are determined immediately and at several times thereafter. This allows assessment of the effects of different chemical concentrations on sludge actually present in the reactor, and evaluation of extended exposure to the chemical in question, as contrasted with determining only the acute impact measured by the original procedure.

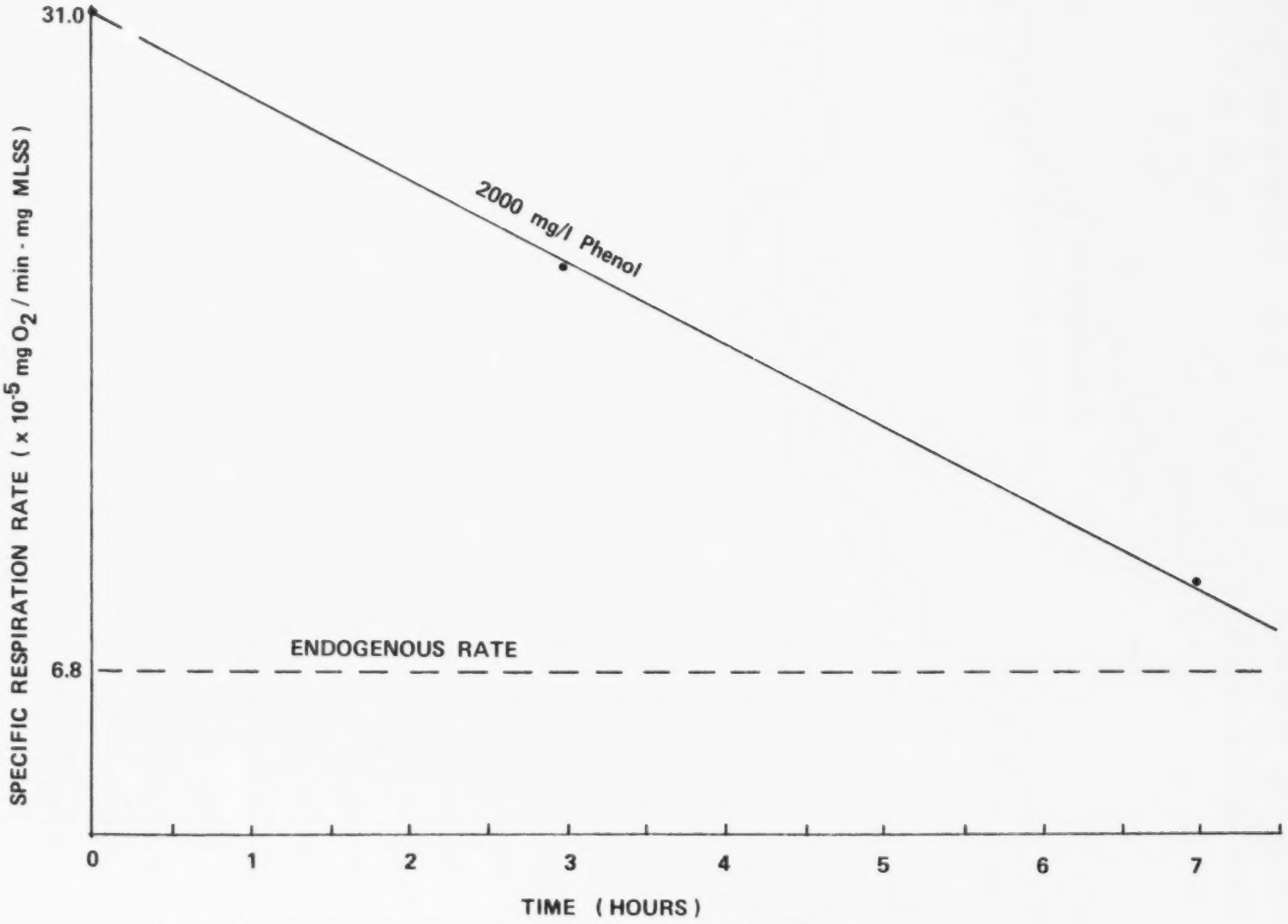


Figure 69. Effect of time on respiration rate with 2000 mg/l phenol.

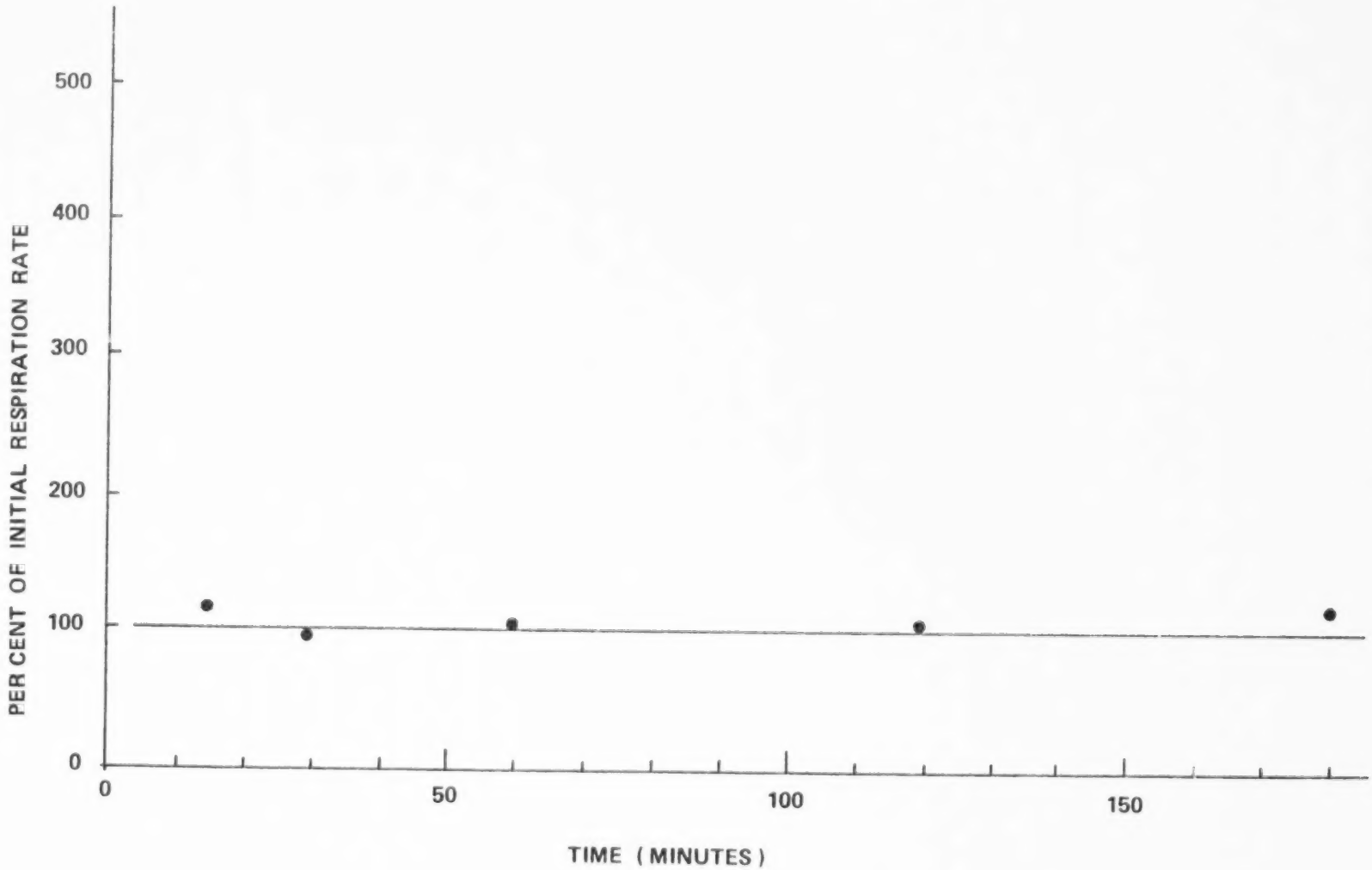


Figure 70. Respiration rate of "fresh" sludge from reactor.

Figure 71 shows results obtained using the new procedure, with phenol as the substrate. The curve labeled 4/7/79 shows the effect of phenol on the respiration rate of "fresh" sludge immediately after phenol addition. The upper curve for runs made on 4/11/79 agrees reasonably well with data for corresponding concentrations on the earlier curve, illustrating reproducibility.

The lower curves in Figure 71 on 4/11/79 indicate respiration rates of the samples after 3, 5 and 30 hours of continuous aeration without feed. The curve for time zero shows no toxicity even at 100% of the feed concentration (2,000 mg/l). Data after several hours of aeration show significant toxicity at 2,000 mg/l phenol, the extent of inhibition increasing with time. The points for 400 mg/l show decreases in respiration rates with time of aeration, which could indicate slight inhibition or, perhaps, exhaustion of the phenol supply in the sample. Clearly, the conclusion which one would reach about the effect of phenol is radically different after several hours of aeration from that observed immediately after its addition to the sample. Apparently, the inhibitory action of phenol is exerted over a period of several hours.

Figure 72 shows results obtained using the modified procedure with 3,5-xylenol, 2-methylquinoline, acetic acid and the synthetic wastewater. All of them indicate utilization by the mixed culture in the reactor and none show immediate inhibition within concentration ranges employed in the tests. It is especially significant that the composite synthetic wastewater does not show any inhibitory effects, even at concentrations twice as high as those of the synthetic formulation. However, it must be recognized that inhibition may become evident subsequently, as shown in

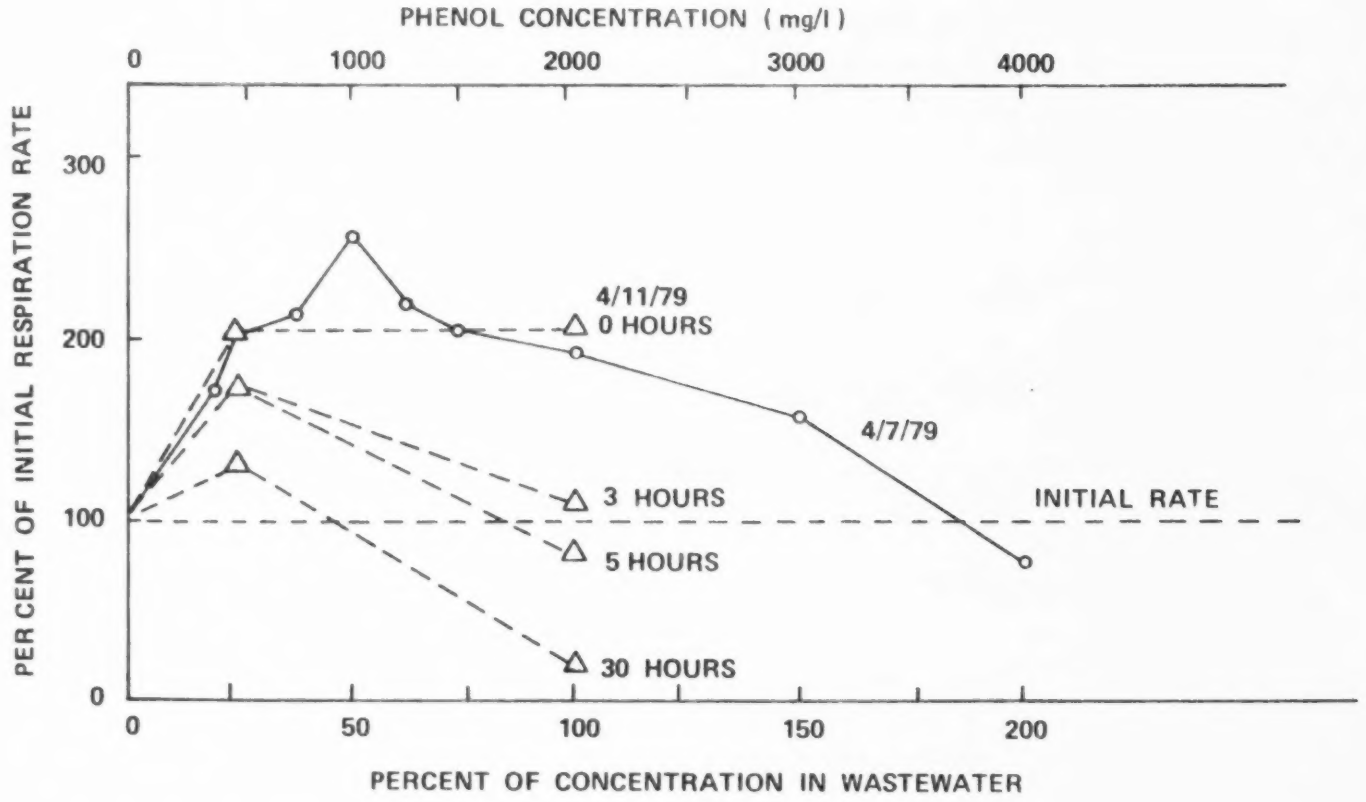


Figure 71. Respiration curves for phenol using modified oxygen utilization procedure.

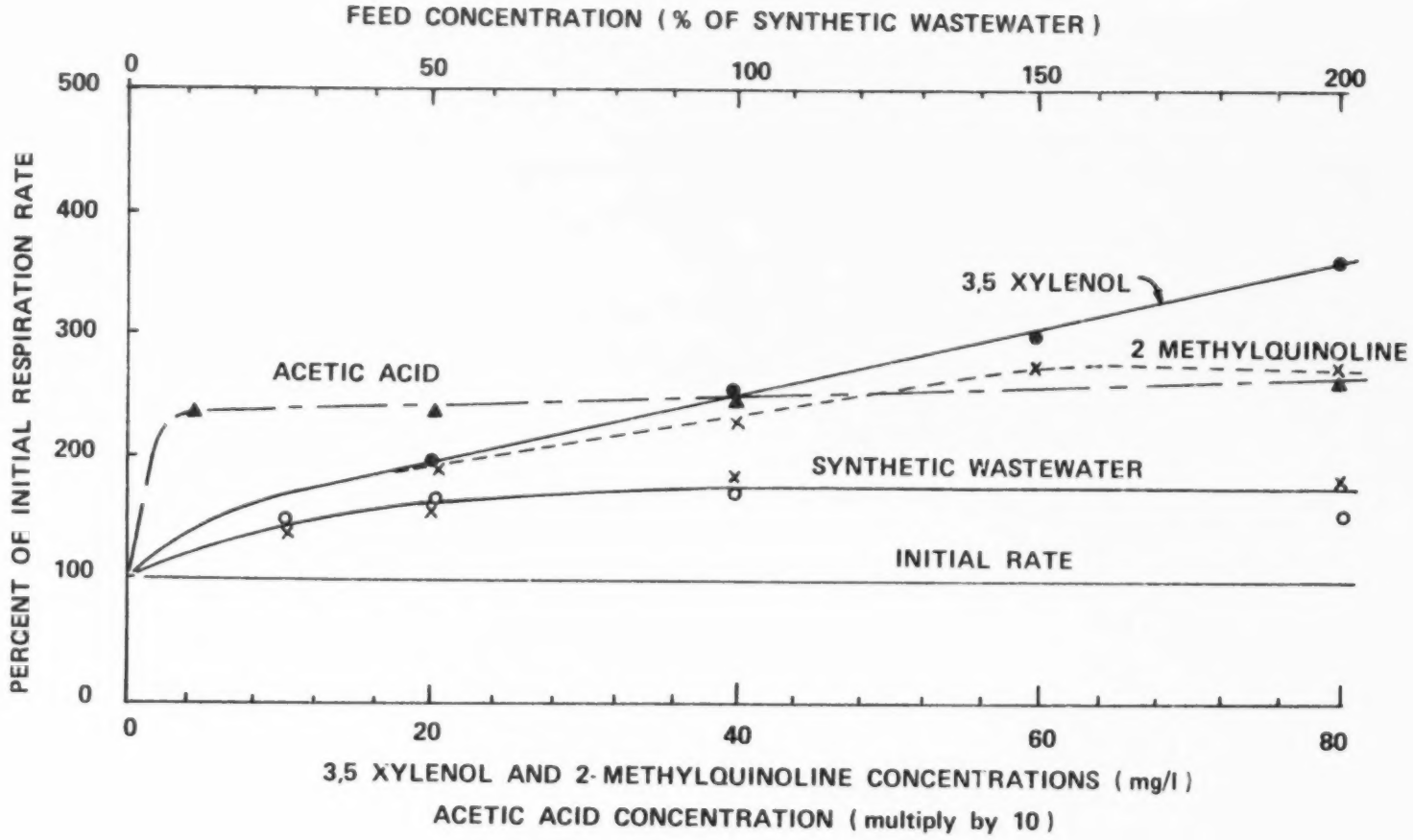


Figure 72. Results of using modified oxygen utilization procedure on selected substrates.

Figure 71 for the curves developed on 4/11. This situation indicates a need for checking the effect of substrate concentration at two or more times, instead of only immediately after addition to the culture.

Evaluation of all chemicals in the synthetic wastewater are now being repeated, using the modified procedure. Within the next few months, the studies will be extended to evaluate the effects of other wastewater constituents not presently included in the synthetic mixture, including: cyanide, thiocyanate, ammonia, and selected priority pollutants. Also, respiration measurements are being used periodically to monitor metabolic activity in the experimental activated sludge reactors.

SECTION 11

ADSORPTION OF ALKYL PHENOLS AND RESIDUAL TOC FOLLOWING BIOLOGICAL TREATMENT

Adsorption by activated carbon or spent char from the coal conversion process itself might be considered to be an integral part in the overall scheme for treatment of coal conversion wastewaters. Adsorption processes might be applied to the adsorbable organic constituents in the raw wastewater, thereby lessening the organic load applied to the biological processes. Alternatively, adsorption processes might be utilized in a post-treatment mode, following biological treatment, in order to remove residual organic substances which are non-biodegradable, or which are not removed as a consequence of the conditions under which the biological treatment units are operated. While there is a significant body of knowledge concerning the adsorption of phenol by activated carbon, little information is available concerning the relative adsorbability of the alkyl phenols and of the other organic constituents of coal conversion wastewaters. Accordingly, the objectives of this portion of the overall investigation of coal conversion wastewater treatability and assessment were :

1) to evaluate the effect of alkyl substitution on the adsorption of phenols by activated carbon, with particular attention directed at the position, length, and number of the alkyl substituents; and

2) to evaluate the adsorbability of the residual TOC following biological treatment.

PROCEDURES

A series of batch adsorption experiments was conducted to evaluate the equilibrium characteristics for the adsorption of phenol and several alkyl phenols on powdered activated carbon. The experiments were carried out on a single component basis, using phenol, 2-, 3-, and 4-methylphenol (cresols), several isomers of dimethyl- (xylenols) and ethylphenols, and 2-isopropylphenol. All chemicals were reagent-grade, from Aldrich Chemical Company, and were used as received. PX-21, a powdered activated carbon from Amoco Research Corporation, Naperville, Illinois, with a specific surface area of 2,800 to 3,500 m^2/gm, was used as the adsorbent.

Various dilutions, ranging from 20 to 60 mg/l, of stock phenolic adsorbate solutions were addded to different amounts of activated carbon, ranging from 540 to 1,000 mg/l. The suspensions were mixed for four hours, at room temperature, on a Phipps and Bird six-place gang stirrer. (Preliminary experimentation assured that equilibrium was attained within four hours.) Aliquots of the suspension were removed at the end of this contact period and centrifuged to remove the powdered carbon. The UV absorbance of the resulting concentrate was measured using a Varian Techtron Model 635 spectrophotometer, employing the UV absorbance maxima listed in Table 25. Comparison of the absorbance measurements to a characteristic calibration curve for the substrate in question allowed for calculation of the equilibrium adsorbate concentration.

TABLE 25. CHARACTERISTIC WAVELENGTHS FOR MAXIMUM UV ABSORBANCE OF AQUEOUS PHENOLIC SOLUTIONS

Compound	Wavelength, (nm)
Phenol	269
2-Methylphenol	270
3-Methylphenol	271
4-Methylphenol	276.5
2-Ethylphenol	270
3-Ethylphenol	270.2
4-Ethylphenol	274
2, 3-Dimethylphenol	271
3, 4-Dimethylphenol	274
2, 6-Dimethylphenol	268
2-Isopropylphenol	269.5

For the studies directed at the adsorption of the residual TOC from the biological treatment units, Nuchar WV-G, a granular activated carbon provided by the Westvaco Chemical Co., Covington, W. Va., was used as the adsorbent. The carbon was obtained from the manufacturer in crushed form, and was washed twice with distilled water, dried at 200^{o}C in an oven for 24 hours, and allowed to cool in a dessicator where it was subsequently stored until it was used.

Effluent samples from the biological reactors during the second series of steady-state studies were collected, centrifuged, and filtered through Gelman glass fiber filters (type A/E) with a pore size range of 0.2 to 10 μm to remove suspended material and microorganisms. Batch equilibrium adsorption studies were conducted on the fresh filtrate, without any dilution of the samples. A parallel sample of the fresh raw synthetic wastewater was also included in the adsorption studies. Various amounts of activated carbon, ranging from 100 to 5,000 mg/l were added to the filtered effluent samples, and the suspensions were mixed overnight, at room temperature, on a Phipps and Bird six-place gang stirrer to assure that equilibrium had been reached.

At the end of the contact period, the suspensions were allowed to settle for 24 hours while the beakers were covered with Parafilm sheets to minimize changes in concentration due to evaporation and volatilization. Aliquots of the supernatant liquid were filtered through a combination of Whatman GF/D and GF/F glass fiber filter papers, with a maximum pore size of 0.7 μm, in order to remove all fine carbon particles. The filtered samples were stored under refrigeration and ultimately analyzed on a Beckman 915 Carbon Analyzer. Before injection into the TOC analyzer, approximately 3 ml of each sample was acidified with 1 drop of 6N HCl to convert inorganic carbon to CO_2 which was removed by purging with helium. Precipitation of a small amount of material was noted as a result of the acidification step. A 30 μl aliquot of each of the acidified, purged samples was injected into the TOC analyzer and the residual TOC concentration was determined.

RESULTS

Alkyl Phenol Adsorption

Figure 73A is a plot of the data for the adsorption of 2-, 3-, and 4-methylphenol on PX-21. The data conform to the Langmuir adsorption isotherm

$$q_e = (bQ^oC)/(1+bC) \quad (10)$$

where

q_e = quantity of substrate adsorbed per unit weight of adsorbent, in gm/m

C = equilibrium concentration of substrate in solution, in mg/l

b = constant characterizing strength of substrate-adsorbent bond, in $(mg/l)^{-1}$

Q^o = adsorptive capacity in gm/gm

Figure 73B is a plot of the same data in accordance with the linearized form of the Langmuir isotherm

$$C/q_e = (1/Q^o)C + 1/bQ^o \quad (11)$$

yielding the coefficients b = 0.172 $(mg/l)^{-1}$ and Q^o = 0.383 gm/gm. Both plots indicate that position of the substituted methyl group appears to have no effect on the extent of methylphenol adsorption. Figures 74A and 74B, and 75A and 75B, which show similar data for several isomers of dimethylphenol and ethylphenol, respectively, also support this argument.

Composite plots of the adsorption isotherms for phenol, the methyl, dimethyl, and ethylphenols, and 2-isopropylphenol are shown in Figures 76A and 76B. The curves for the methyl, dimethyl , and ethylphenols are developed from Figures 73, 74, and 75, respectively, while the curves for phenol and 2-isopropylphenol are derived from the data illustrated in

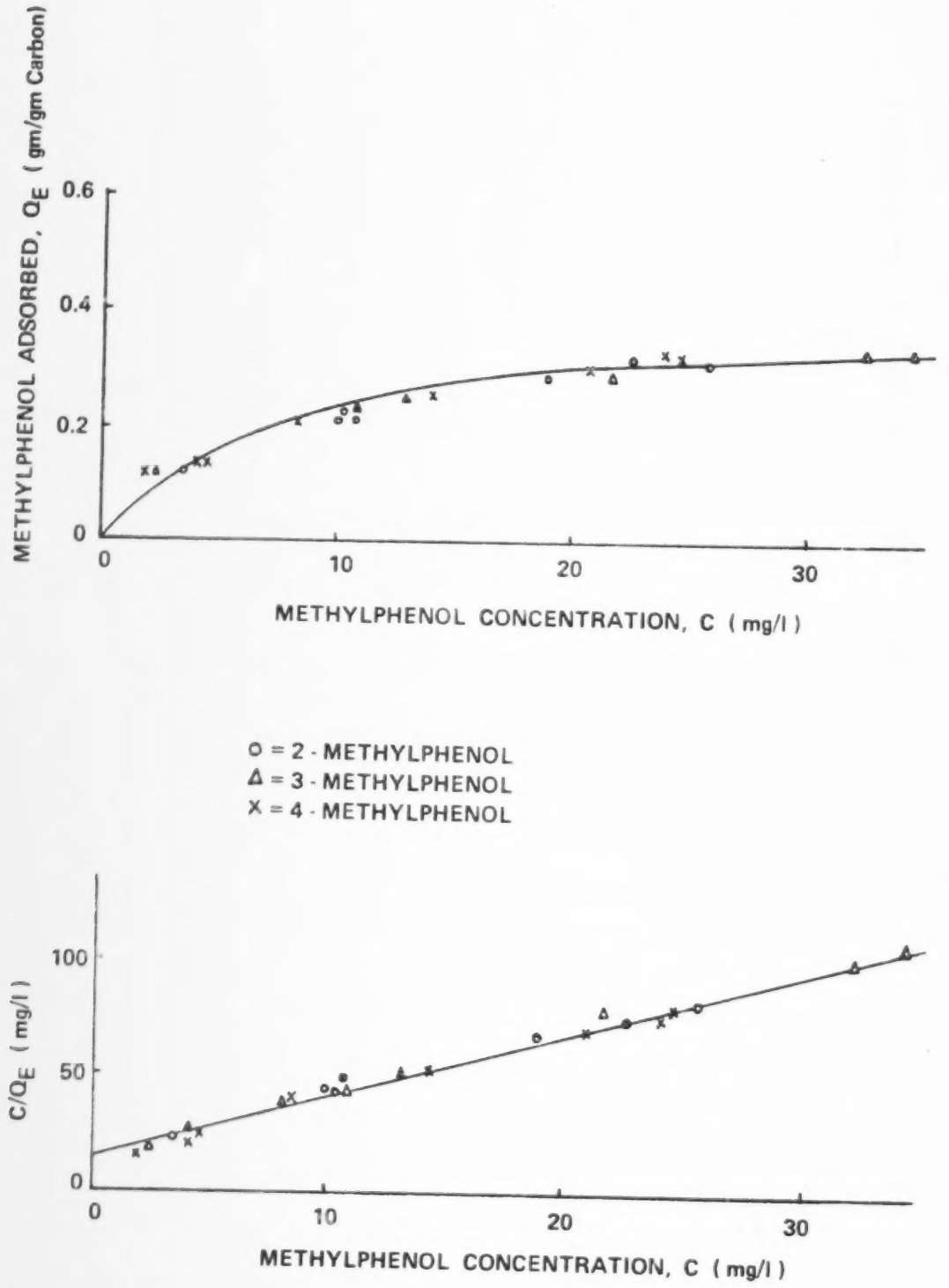
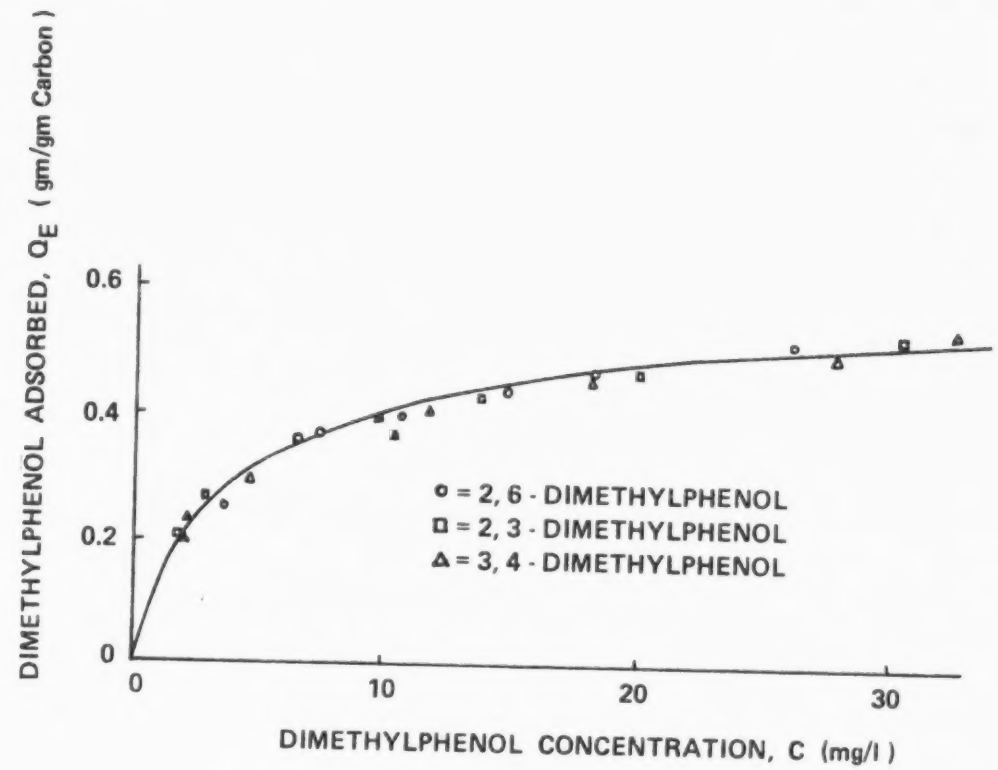


Figure 73. Adsorption of methylphenols by activated carbon. (A.) Uptake as a function of methylphenol concentration. (B.) Linearized Langmuirian relationship.



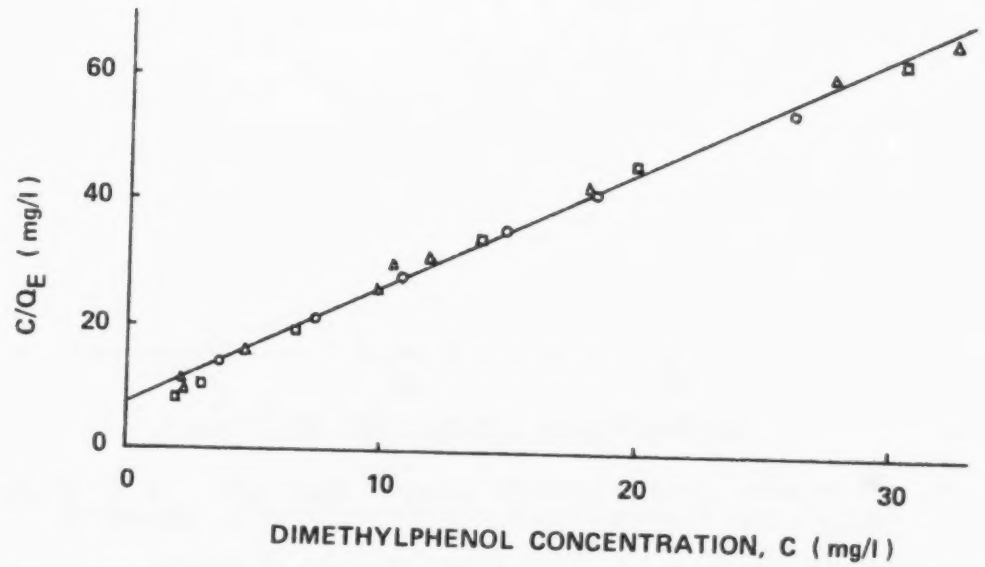


Figure 74. Adsorption of dimethylphenols by activated carbon. (A.) Uptake as a function of dimethylphenol concentration. (B.) Linearized Langmuirian relationship.

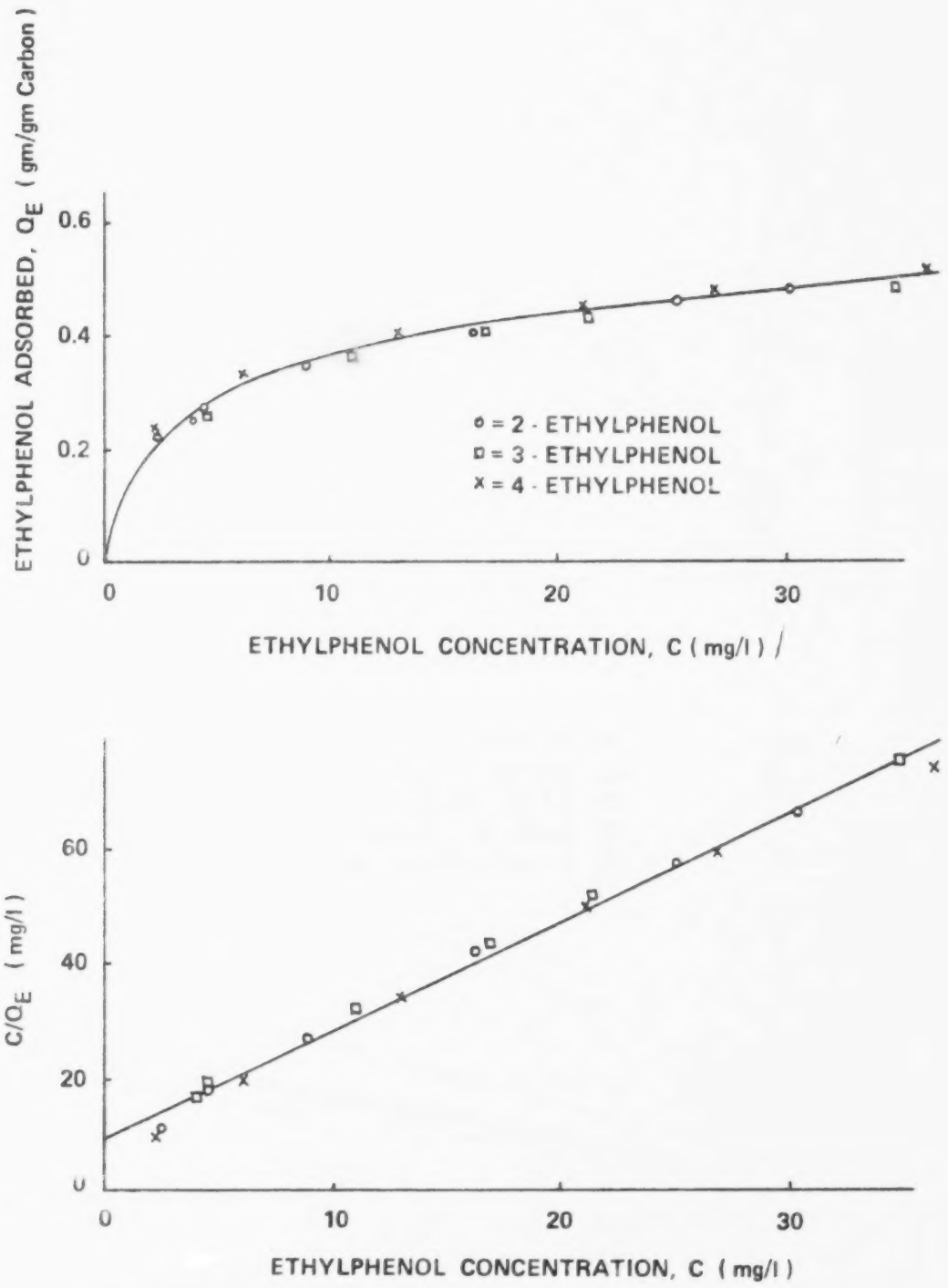


Figure 75. Adsorption of ethylphenols by activated carbon. (A.) Uptake as a function of ethylphenol concentration. (B.) Linearized Langmuirian relationship.

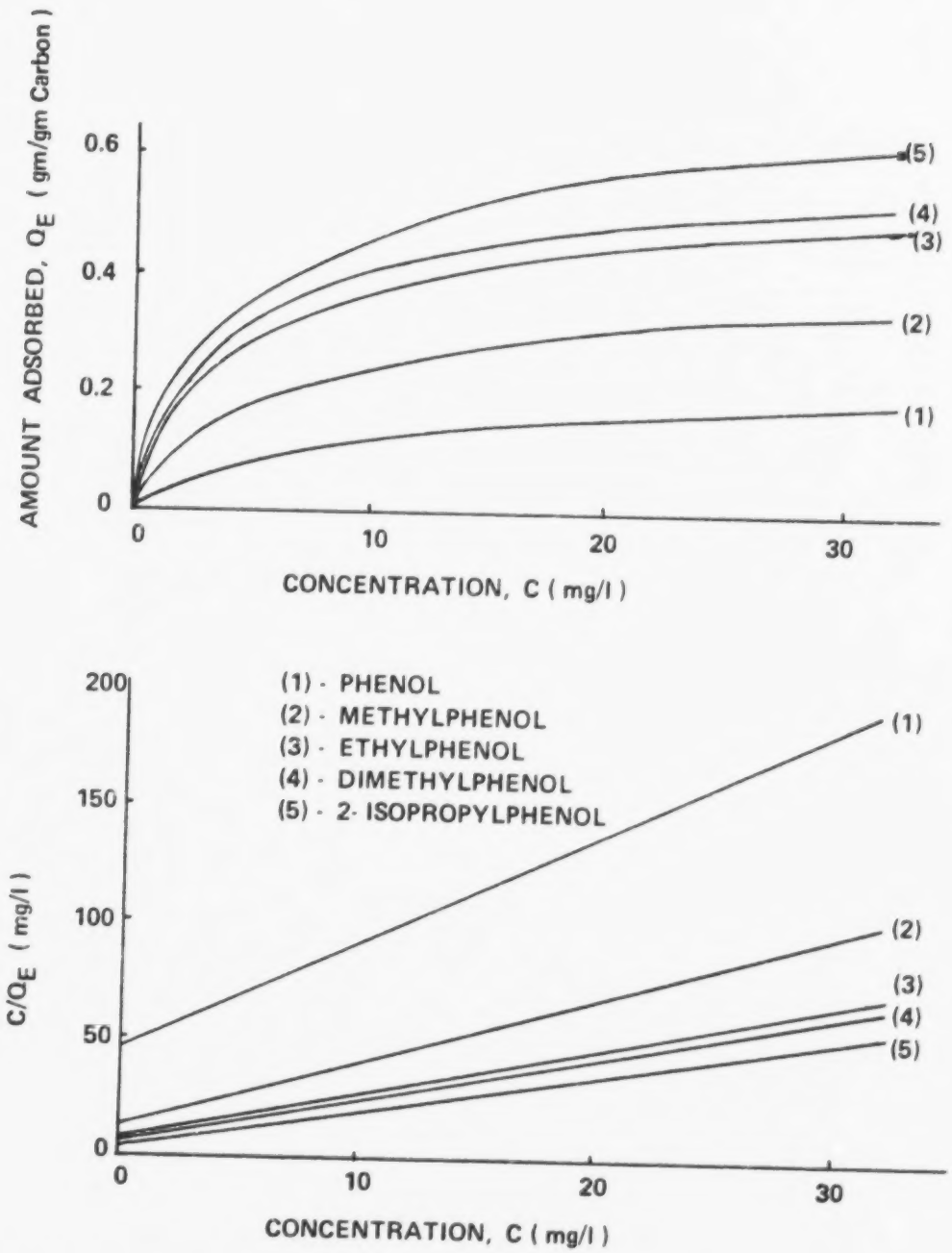


Figure 76. Comparative adsorption of alkyl-substituted phenols by activated carbon. (A.) Uptake as a function of alkyl phenol concentration. (B.) Linearized Langmuirian relationship.

Figures 77 and 78, respectively. The alkyl-substituted phenols are more strongly adsorbed than phenol itself, and adsorption increases as the length of the alkyl chain increases. While position of the substituted alkyl groups has no effect on the extent of adsorption, adsorption is enhanced when the number of substituents on the phenol molecule is increased. Table 26 summarizes the Langmuirian coefficients for the alkyl phenols investigated, both on a weight and molar basis.

The experimental results are in accord with adsorption theory and with the results of previous investigators who have reported that the extent of adsorption increases as the solubility of the adsorbate decreases. The substitution of alkyl groups on the phenol molecule would be expected to make the resulting alkyl phenol less polar and therefore less soluble in water.

Adsorbability of Biologically-Treated Effluent

For a mixture of organic compounds, adsorption equilibria can usually be described by the Freundlich isotherm

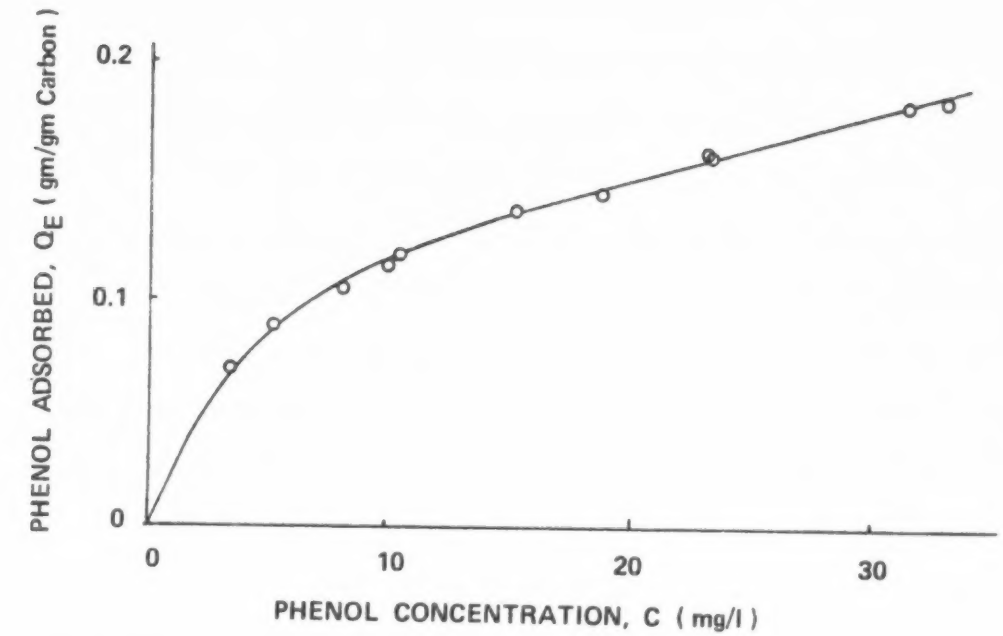
$$q_e = kC^{1/n} \tag{12}$$

where q_e and C are, respectively, the quantity of organic material (e.g. TOC) adsorbed per unit weight of adsorbent (activated carbon), and the residual equilibrium concentration of organic material (TOC) in solution. "k" and "n" are constants. The Freundlich relationship can be rearranged to give

$$\log q_e = \log k + 1/n \log C \tag{13}$$

which yields a linear plot when $\log q_e$ is plotted against log C.

Figure 79 presents a linearized Freundlich plot showing the adsorbability of the TOC comprising the raw synthetic wastewater and the



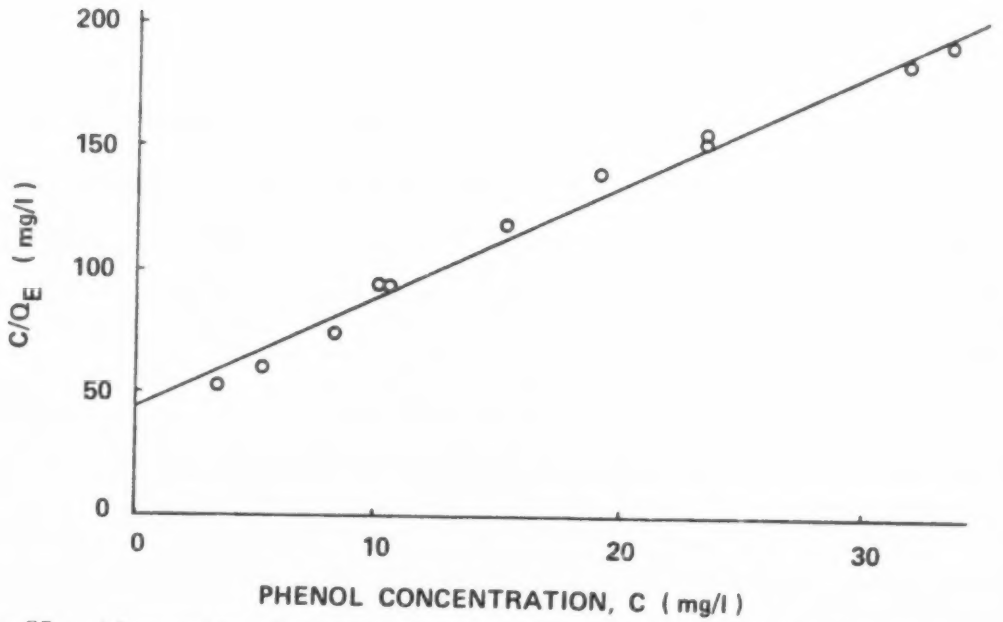


Figure 77. Adsorption of phenol by activated carbon. (A.) Uptake as a function of phenol concentration. (B.) Linearized Langmuirian relationship.

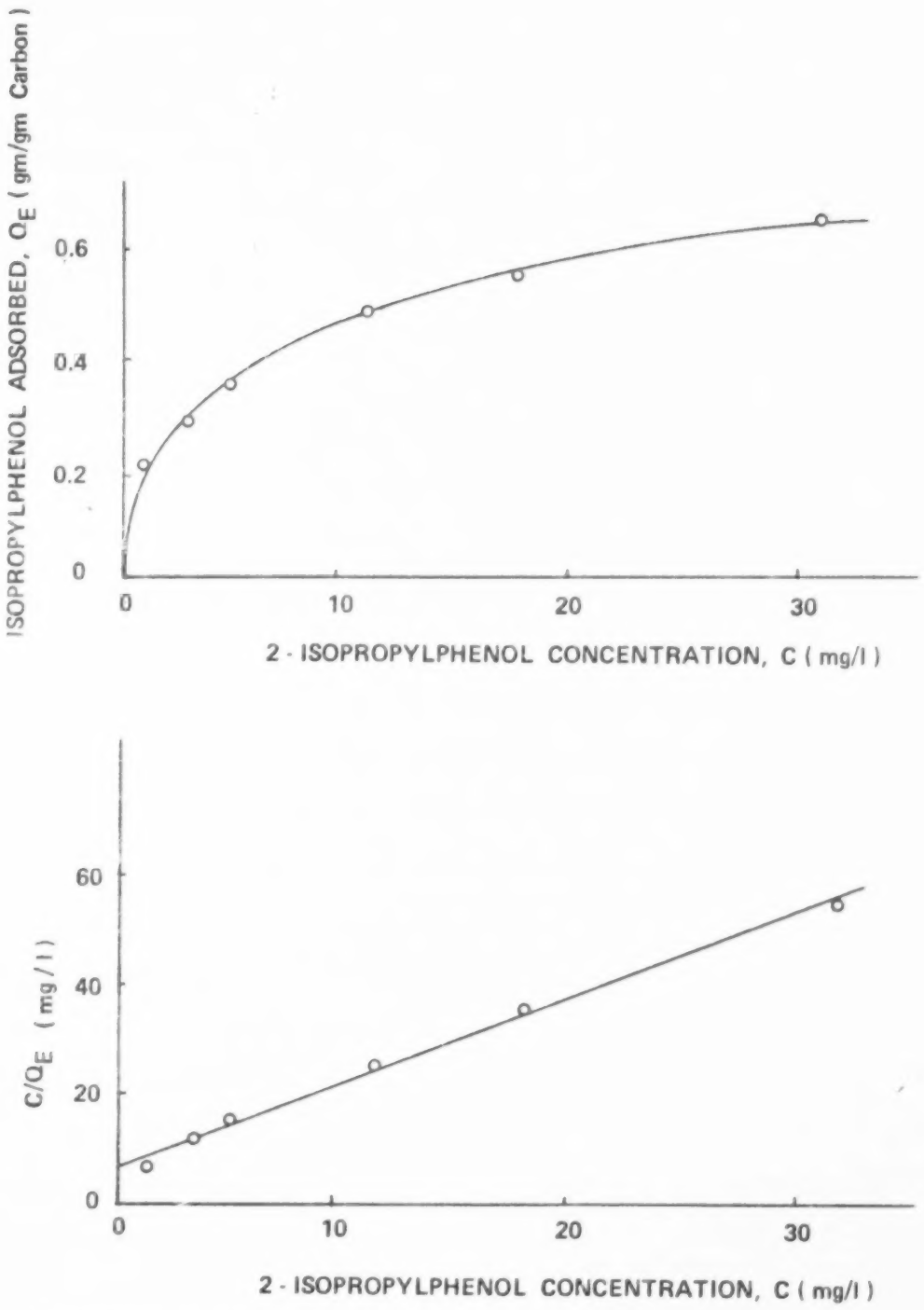


Figure 78. Adsorption of isopropylphenol by activated carbon. (A.) Uptake as a function of isopropylphenol concentration. (B.) Linearized Langmuirian relationship.

TABLE 26. LANGMUIRIAN COEFFICIENTS FOR THE ADSORPTION OF ALKYL PHENOLS ON PX-21 POWDERED ACTIVATED CARBON

Substrate	b $(mg/l)^{-1}$	Q^o gm/gm	b $(mmole/l)^{-1}$	Q^o (mmole/gm carbon)
Phenol	0.0878	0.234	8.26	2.49
2-Methylphenol 3-Methylphenol 4-Methylphenol	0.172	0.383	18.6	3.54
2-Ethylphenol 3-Ethylphenol 4-Ethylphenol	0.222	0.532	27.1	4.35
2,3-Dimethylphenol 2,6-Dimethylphenol 3,4-Dimethylphenol	0.248	0.552	30.3	4.52
2-Isopropylphenol	0.245	0.671	33.6	4.89

biologically-treated effluent from the 5-, 20-, and 40-day reactors. At this time, these results can only be considered to be preliminary but they do suggest that the adsorbability of the residual organic constituents following biological treatment decreases with increasing extent of biological treatment. This does not imply that the residual organics cannot be removed to a further degree by adsorption on activated carbon, but it does indicate that the solid/solution equilibrium is shifted such that a greater amount of adsorbent would be required to achieve a given level of TOC removal.

These results are not surprising in view of the previous discussion in Section 7 relating to the relative polarity of the residual organics comprising the effluent TOC from the biological reactors. High performance

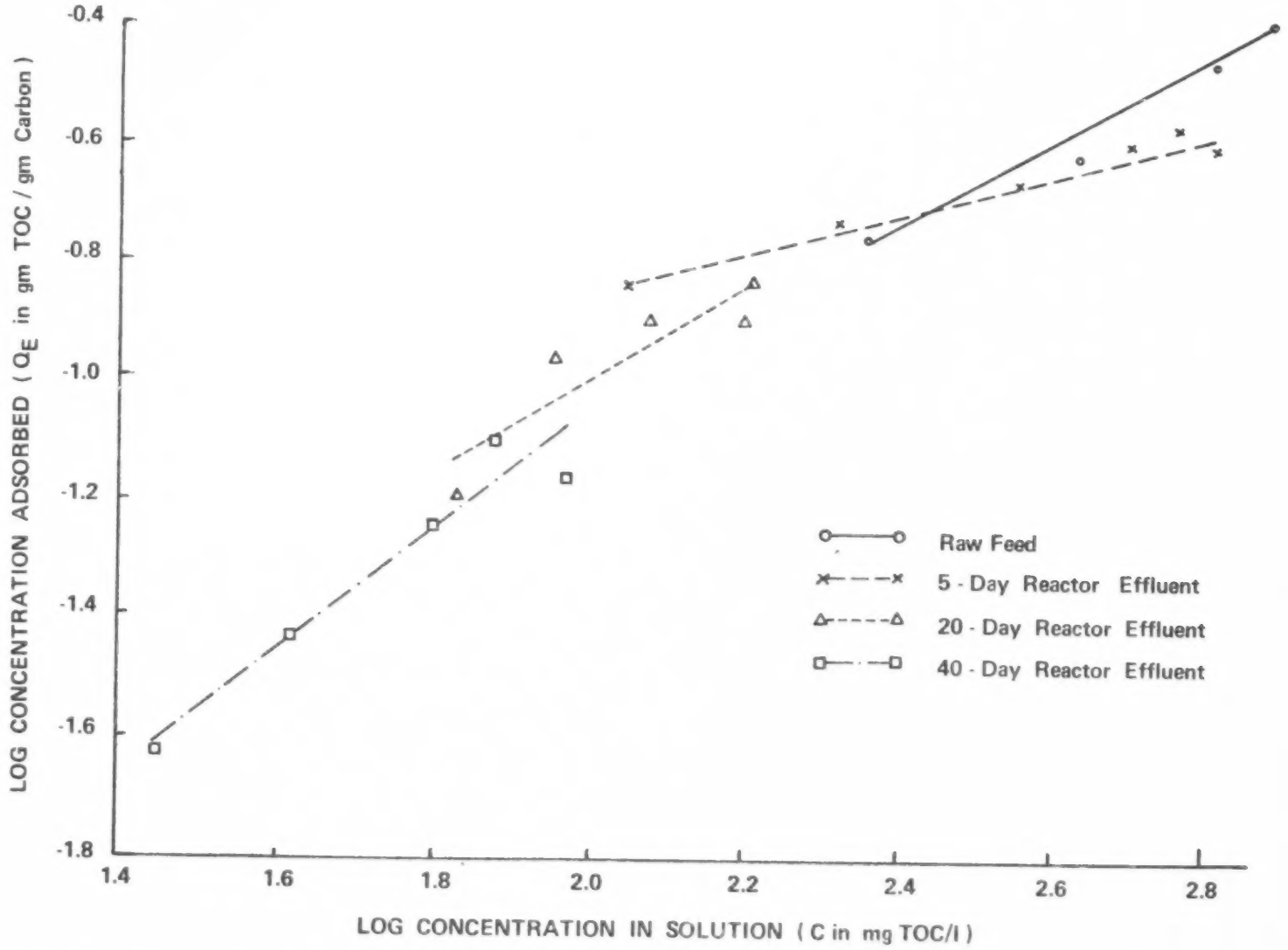


Figure 79. Adsorption of raw and biologically-treated synthetic wastewater by activated carbon.

liquid chromatographic analyses demonstrated that biological treatment, while substantially reducing the overall concentration of TOC, resulted in the production of a sizeable amount of polar material, presumed to be biological metabolites of the biochemical reactions taking place. The increased polarity of the residual TOC would tend to make the organics more soluble in water and therefore less adsorbable by a hydrophobic substance like activated carbon.

These adsorbability studies are continuing in order to define better the response of the residual TOC in the biologically-treated effluent to adsorption by activated carbon and spent char.

REFERENCES

1. Singer, P. C., F. K. Pfaender, J. Chinchilli, A. F. Maciorowski, J. C. Lamb III, and R. Goodman. Assessment of Coal Conversion Wastewaters: Characterization and Preliminary Biotreatability. Report No. EPA-600/7-78-181, U. S. Environmental Protection Agency, Washington, DC (September 1978).

2. Forney, A. J., W. P. Haynes, S. J. Gasior, G. E. Johnson, and J. P. Strakey. Analysis of Tars, Chars, Cases and Water in Effluents from the Synthane Process. U. S. Bureau of Mines Technical Progress Report 76. Pittsburgh Energy Research Center, Pittsburgh, PA (1974).

3. Luthy, R. G. and J. T. Tallon. Biological Treatment of Hygas Coal Gasification Wastewater. FE-2496-43, U. S. Department of Energy, Washington, DC (December 1978).

4. Johnson, G. E., R. D. Neufeld, C. J. Drummond, J. P. Strakey, W. P. Haynes, J. D. Mack, and T. J. Valiknac. Treatability Studies of Condensate Water from Synthane Coal Gasification. Report No. PERC/RI-77/13, U. S. Department of Energy, Pittsburgh Energy Research Center, Pittsburgh, PA (1977).

5. Reap, E. J., G. M. Davis, J. H. Koon, and M. J. Duffy. Wastewater Characteristics and Treatment Technology for the Liquification of Coal Using the H-Coal Process. Proceedings of 32nd Purdue Industrial Waste Conference, Ann Arbor Science Publishers, Ann Arbor, MI (1977), pp. 929-943.

6. Scott, C. D., C. W. Hancher, D. W. Holladay, and G. B. Dinsmore. A Tapered Fluidized-Bed Bioreactor for Treatment of Aqueous Effluents from Coal Conversion Processes. Proceedings of Second Symposium on Environmental Aspects of Fuel Conversion Technology, EPA-600/2-76-149 (June 1976).

7. Kostenbader, P. D. and J. W. Flecksteiner. Biological Oxidation of Coke Plant Weak Ammonia Liquor. J. Water Pollution Control Federation, 41(2):199-207 (February 1969).

8. American Public Health Association. Standard Methods for the Examination of Water and Wastewater, 14th ed. Washington, DC (1975).

9. Luthy, R. G. Manual of Methods: Preservation and Analysis of Coal Gasification Wastewaters. U. S. Department of Energy, DOE FE-2496-8 (July 1977).

10. O'Melia, C. R. Coagulation and Flocculation. Chapter 2 in Physiochemical Processes for Water Quality Control (W. J. Weber, Jr., ed.). Wiley and Sons, New York (1972), pp. 61-109.

11. Metcalf and Eddy. Wastewater Engineering. McGraw Hill Book Co. (1972).

12. Drummond, C. J., G. E. Johnson, R. D. Neufeld and W. P. Haynes. Biochemical Oxidation of Coal Conversion Wastewaters. Presented at 87th National Meeting of Amrican Institute of Chemical Engineers, Boston, MA (August 1979).

13. Duke, K. M., M. E. Davis, and A. J. Dennis. IERL-RTP Procedures Manual: Level I Environmental Assessment. Biological Tests for Pilot Studies, EPA-600/7-77-043, U. S. Environmental Protection Agency (April 1977).

14. Umbreit, W. W., R. H. Burris, and J. F. Stauffer. Manometric Techniques. Burgess Publishing Co., Minneapolis, MN (1964).

15. Alexander, M. and B. K. Lustigman. Effect of Chemical Structure on Microbial Degradation of Substituted Benzenes. J. Agric. Food Chem., 14:410-413 (1966).

16. Lamb, J. C., W. C. Westgarth, J. L. Rogers, and A. P. Vernimmen. A Technique for Evaluating the Biological Treatability of Industrial Wastes. J. Water Pollution Control Federation, 36:1263-1284 (1964).

17. Jenkins, D. The Use of Manometric Methods in the Study of Sewage and Trade Wastes. Page 99 in Waste Treatment. Pergamon Press, New York (1960).

18. Gellman, I. and H. Heukelekian. Biological Oxidation of Formaldehyde. Sewage and Industrial Wastes, 22(10):1321 (October 1950).

19. Skrinde, R. T. and C. N. Sawyer. Application of the Warburg Respirometer to Industrial Waste Analysis - Special Studies on Radioactive Wastes. Proceedings of the 7th Industrial Wastes Conference, Purdue University Extension Service (1952), p. 481.

20. Dickerson, B. W., C. J. Campbell, and M. Stankard. Further Operating Experiences on Biological Purification of Formaldehyde Wastes. Proceedings of the 9th Industrial Wastes Conference, Purdue University Extension Service (1954), p. 331.

21. Stack, V. T. and R. A. Conway. Design Data for Completely Mixed Activated Sludge Treatment. Sewage and Industrial Wastes, 31(10):1181 (October 1959).

22. Wilson, I. S. The Monsanto Plant for the Treatment of Chemical Wastes. J. and Proceedures, Inst. Sew. Purif. (Brit.), 86 (1954).

23. Lamb, J. C. Reports on Industrial Waste Investigations. Unpublished reports prepared for a chemical industry. (1962, 1963).

24. Matthews, L. A. Application of the Oxygen Electrode to Problems of Waste Treatability. Proc. 13th S. Mun. and Ind. Waste Conf., Duke University (in press).

25. Heukelekian, H. and M. C. Rand. Biochemical Oxygen Demand of Pure Organic Compounds. Sewage and Industrial Wastes, 27:1040-1050 (1955).